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FATAL FULMINANT ACUTE CARBON TETRACHLORIDE POISONING — ROBERT B. JENNINGS, M.D.

CHOLESTEROL CONTENT OF AORTA IN RELATION TO SEVERITY OF ATHEROSCLEROSIS — GARDNER C. McMILLAN, M.D., ET AL.

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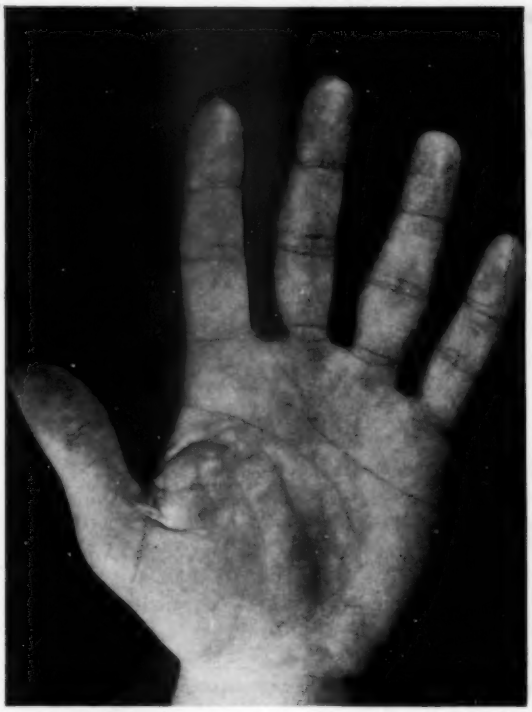

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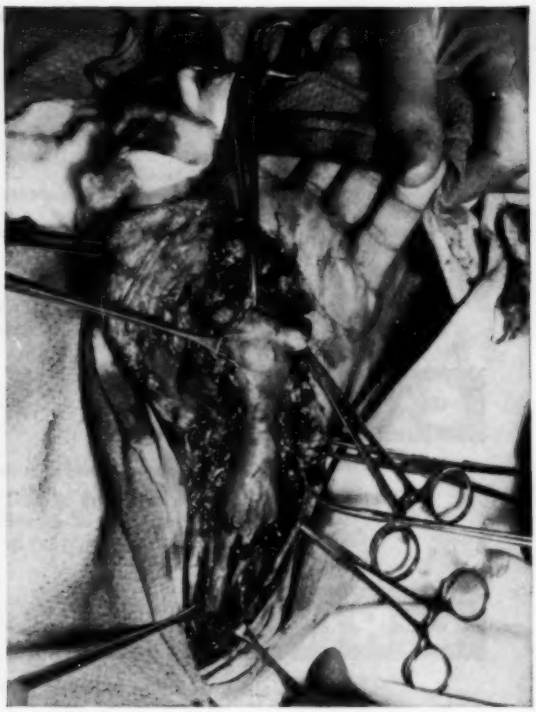

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# Gunshot Wounds

BERNHARD STEINBERG, M.D., Toledo, Ohio

Gunshot wounds are among the common causes of suicidal and homicidal deaths. The examining physician, usually a pathologist, assumes a certain degree of responsibility to the law-enforcing agencies for technical information regarding the gunshot wound. A certain amount of data, some of it definite, can be secured from an examination of the area of entrance of the missile, its course, and the point of exit. It is well to keep in mind that the material obtained by the physician is only a part of the total evidence. The ballistic expert has a significant function. The police officials who investigate the circumstances contribute essential data. It is equally as important for the physician to realize the limitations of his findings as it is to find them. Many a positive statement was determined eventually to be false, or else it confused the issues.

The examining physician should have a mental picture of the kind of information expected of him. He should proceed thereafter with his inspection upon that basis and with an established routine. The essential answer that the law-enforcing agencies desire to secure relates to the circumstances responsible for the death. Was it suicidal or homicidal? If neither, was it accidental? These questions may not be answerable until the physician completes the total examination. Even after that, conditions may prevent a positive conclusion. There should be no hesitancy on the part of the medical examiner to state bluntly that he is unable to arrive at a positive diagnosis. Other agencies will step in to secure evidence for a conclusive answer.

There is a definite routine in the examination of the body, the clothing, and the site of the death. Very frequently the physician is presented only with the body. The coroner and the police officials have examined, presumably, either or both the clothing and the site. This information should be supplied to the pathologist so that he may be able to organize all the facts.

The location and the position of the body, preferably with photographs; the relationship of the body to doors and windows; presence of bullet holes in floor, walls, or ceiling, and the slant of the hole represent pertinent information which must be obtained at the presumptive site of death and described by word and sketch. The clothing must be examined for bullet tears, powder, dirt, grease, and blood. The contour of the tear is recorded best by description, drawing, and a photograph. The clothing, as a whole, should be carefully scrutinized for tears and foreign materials.

## EXAMINATION OF THE EXTERNAL BULLET WOUND

A certain amount of factual data must be known by the pathologist so that he may properly interpret the findings. The wound of entry should offer complete, partial, or suggestive information on the following factors:

Distance of muzzle from wound (range)
Direction of the missile (angle)
Diameter of the bullet (caliber)
Relation to life (antemortem or postmortem wound)

Excellent data regarding firearms, ammunition, projectiles, and wounds due to various firearms are available in several

Presented at the seminar of the Ohio State Coroners' Association, Toledo, on May 21-24, 1954.

Director of Laboratories and the Institute of Medical Research, Toledo Hospital; lecturer in forensic medicine to the Police Academy of the City of Toledo.

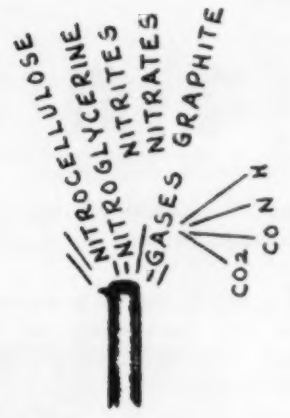


Fig. 1.—Schematic picture of the explosion incident to a bullet leaving the muzzle.

texts.* The examiner should have a general knowledge of firearms, including the caliber of weapons usually employed, the chemical composition of explosive powders, and the mechanism by which a missile is expelled.

When a bullet leaves the muzzle, it is accompanied by gas, flame with smoke, and burned and unburned grains of powder (Fig. 1). Parts of the chemical components of the powder remain in the barrel of the weapon. Other portions may be deposited on the edges of the wound, depending upon the distance of the muzzle. Since the various chemical substances remain in the barrel for varying periods of time, their presence or

* References 1 through 4.

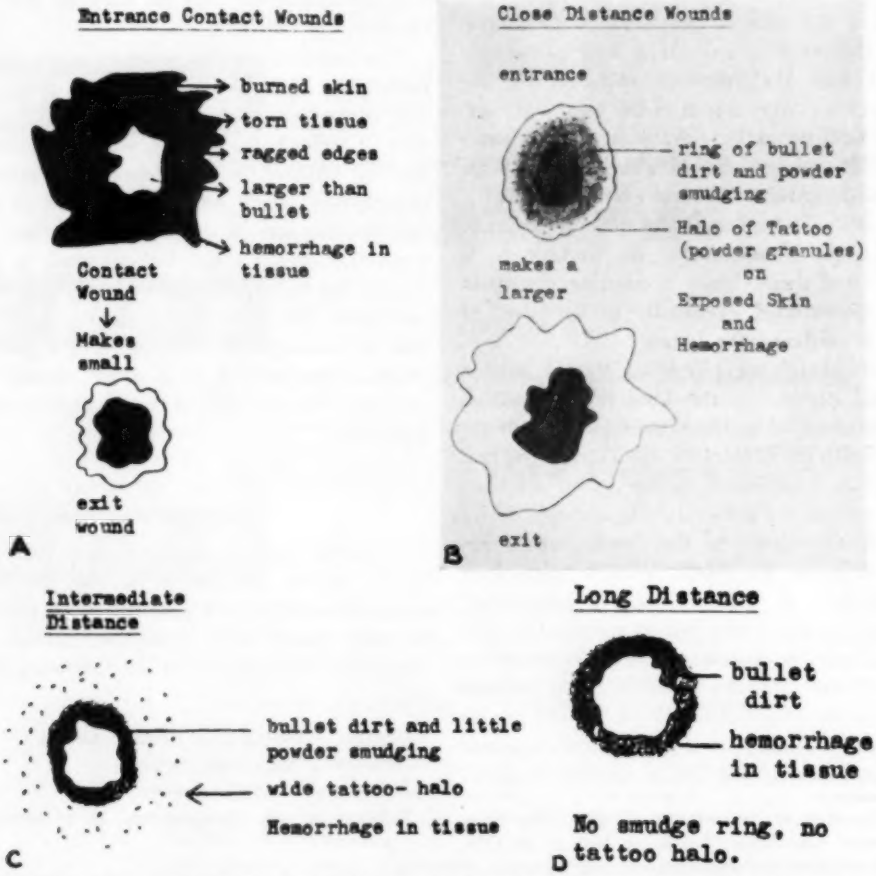


Fig. 2.—Appearance of wounds with muzzle at various distances from point of entry. *A*, contact wound; *B*, wound at close distance; *C*, wound produced at intermediate distance; *D*, wound at long distance. Note the changes in the tissue surrounding the wound at the different distances of firing. The dirt and the smudging can be washed off.

absence may indicate the time of firing. The explosive force of the gases, the flame, the dirt of the missile, and powder grains leave telling evidences which aid in determining the distance of the weapon from the body.

On the basis of the tissue changes induced by firing a missile, distances (range) may be determined with a fair degree of accuracy. If the weapon and ammunition used are available, they should be used to fire test missiles for comparison and a more accurate determination of the range. The following range of distances may be determined by the examination of the wound (Fig. 2):

Contact
Close distance (up to 6 in.)
Intermediate distance (from 6 to 18 in.)
Long distance (over 18 in.)

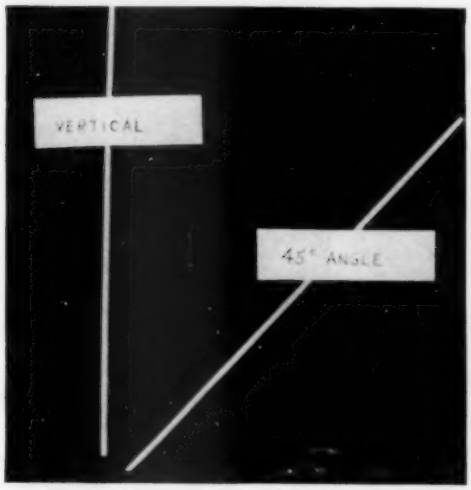


Fig. 3.—Determination of angle of muzzle in relation to the target. A probe placed in the bullet hole gives the angle of fire.

When the muzzle is in contact with the skin, in most cases the explosive force tears the tissue, the flame burns it, smoke blackens it, and blood seeps into it (Fig. 2*A*). The hole is larger than the caliber of the bullet. The exit wound is smaller than the entrance. A contact wound is usually the one condition in which this relationship is true.

Wounds produced on bare skin at a close distance of up to 6 in. show variable degrees of bullet dirt and grease, some coarse tearing of edges, smudging by burning powder, and a halo of exploded and unexploded granules of powder (Fig. 2*B*). There are variable degrees of hemorrhage. The hole is usually smaller than the caliber of the bullet. The point of exit of the bullet is larger than the point of entrance. A bullet penetrating through clothing may not leave dirt, smudge, or powder marks on the skin.

In intermediate distances of 6 to 18 in., the dirt, the smudge, and the halo of powder particles are present but vary in concentration with distance, type of powder, and caliber of bullet (Fig. 2*C*). The halo covers a greater area and is sparse.

When the firearm is held at distances greater than 18 in., the halo of powder granules is usually inconspicuous or absent. The edges show but little tearing. Slight smudging and dirt may be found (Fig. 2*D*). The hole is a neat, penetrating wound.

The direction the missile takes from the muzzle to the target may be determined by the shape of the wound. The location of the powder halo and the smudging are additional aids in ascertaining distances less than 18 in. Another factor is the course the missile takes in the body. If a bullet lodges itself somewhere in the wall, floor, or ceiling of the site of the shooting, a probe inserted into the hole will show the angle of the muzzle (Fig. 3).

A bullet which enters the target horizontally produces a more or less round hole, with a fairly equal degree of tissue tearing throughout the diameter of the wound. Hemorrhages do not show conspicuous variations in quantity along the edges. As a rule, the hemorrhage is distributed fairly evenly along the rim of the hole (Fig. 4*A*). Bullet dirt, smudging, charring of tissue, and the halo of powder granules are fairly equally distributed along the circle of the wound. Wood and plaster may show some undermining because of the splintering effect. This is especially true if the target is within a few

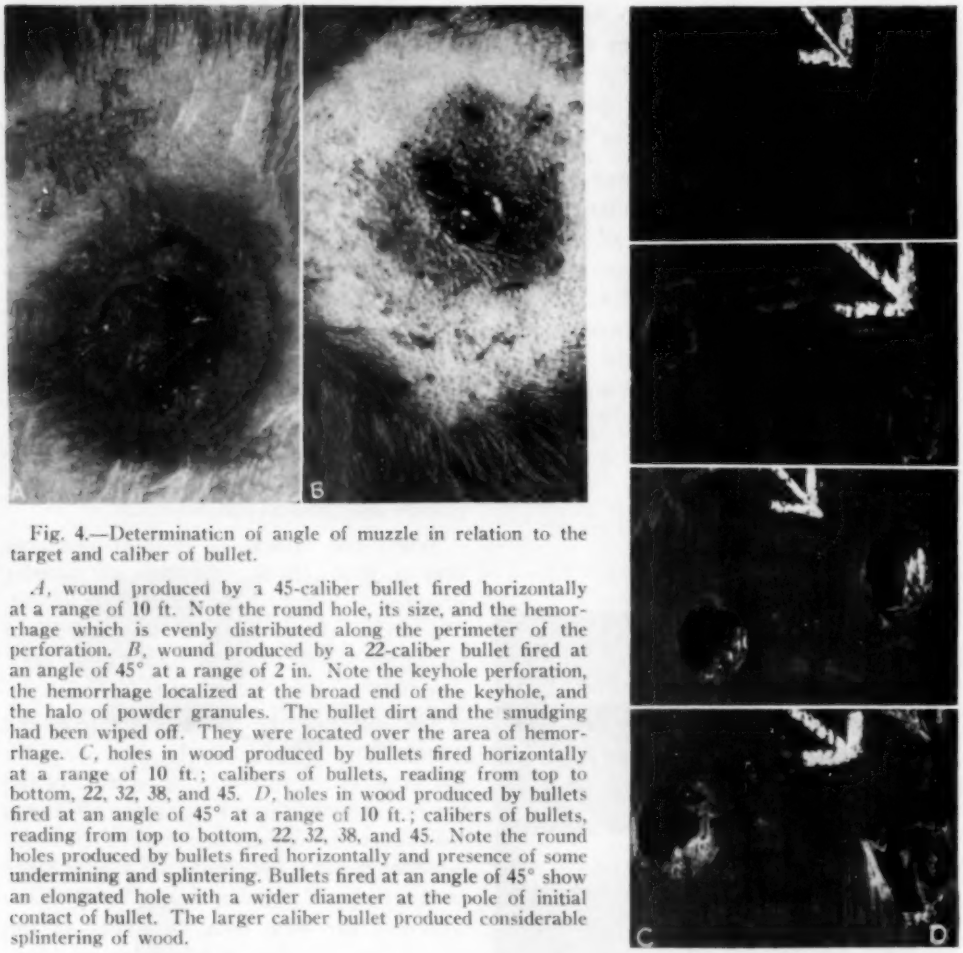

Fig. 4.—Determination of angle of muzzle in relation to the target and caliber of bullet.

*A*, wound produced by a 45-caliber bullet fired horizontally at a range of 10 ft. Note the round hole, its size, and the hemorrhage which is evenly distributed along the perimeter of the perforation. *B*, wound produced by a 22-caliber bullet fired at an angle of 45° at a range of 2 in. Note the keyhole perforation, the hemorrhage localized at the broad end of the keyhole, and the halo of powder granules. The bullet dirt and the smudging had been wiped off. They were located over the area of hemorrhage. *C*, holes in wood produced by bullets fired horizontally at a range of 10 ft.; calibers of bullets, reading from top to bottom, 22, 32, 38, and 45. *D*, holes in wood produced by bullets fired at an angle of 45° at a range of 10 ft.; calibers of bullets, reading from top to bottom, 22, 32, 38, and 45. Note the round holes produced by bullets fired horizontally and presence of some undermining and splintering. Bullets fired at an angle of 45° show an elongated hole with a wider diameter at the pole of initial contact of bullet. The larger caliber bullet produced considerable splintering of wood.

inches of the muzzle and the explosive force of the gases. However, a close inspection and measurement of the outside diameter will reveal a fairly circular opening (Fig. 4*C*).

A missile fired at an angle produces a more or less keyhole type of a wound (Fig. 4*B*). The broad end of the keyhole is the point of initial contact between the bullet and the target. The hemorrhage, the smudging, the burned tissue, and the powder halo, wherever they are present, tend to concentrate at the broad end of the keyhole. Wood and plaster present a similar picture. Despite the tendency to splinter, measurements of the outside diameter will reveal the keyhole shape (Fig. 4*D*).

### CALIBER OF THE BULLET, ENTRANCE AND EXIT WOUNDS, RELATION TO LIFE

It is not always possible to diagnose the caliber of the bullet by the size of the entrance wound. The distance of the muzzle from the target and the angle of firing are some of the factors which interfere with identification (Fig. 5*A*, *B*, *C*, *D*). Since exit wounds are larger than the entrance, except for the contact type, additional difficulties may arise if differentiation between entrance and exit holes becomes uncertain. Holes made in wood or plaster offer less difficulties. Obviously the recovery of the bullet itself establishes its caliber. It is frequently no simple matter to locate the missile in the

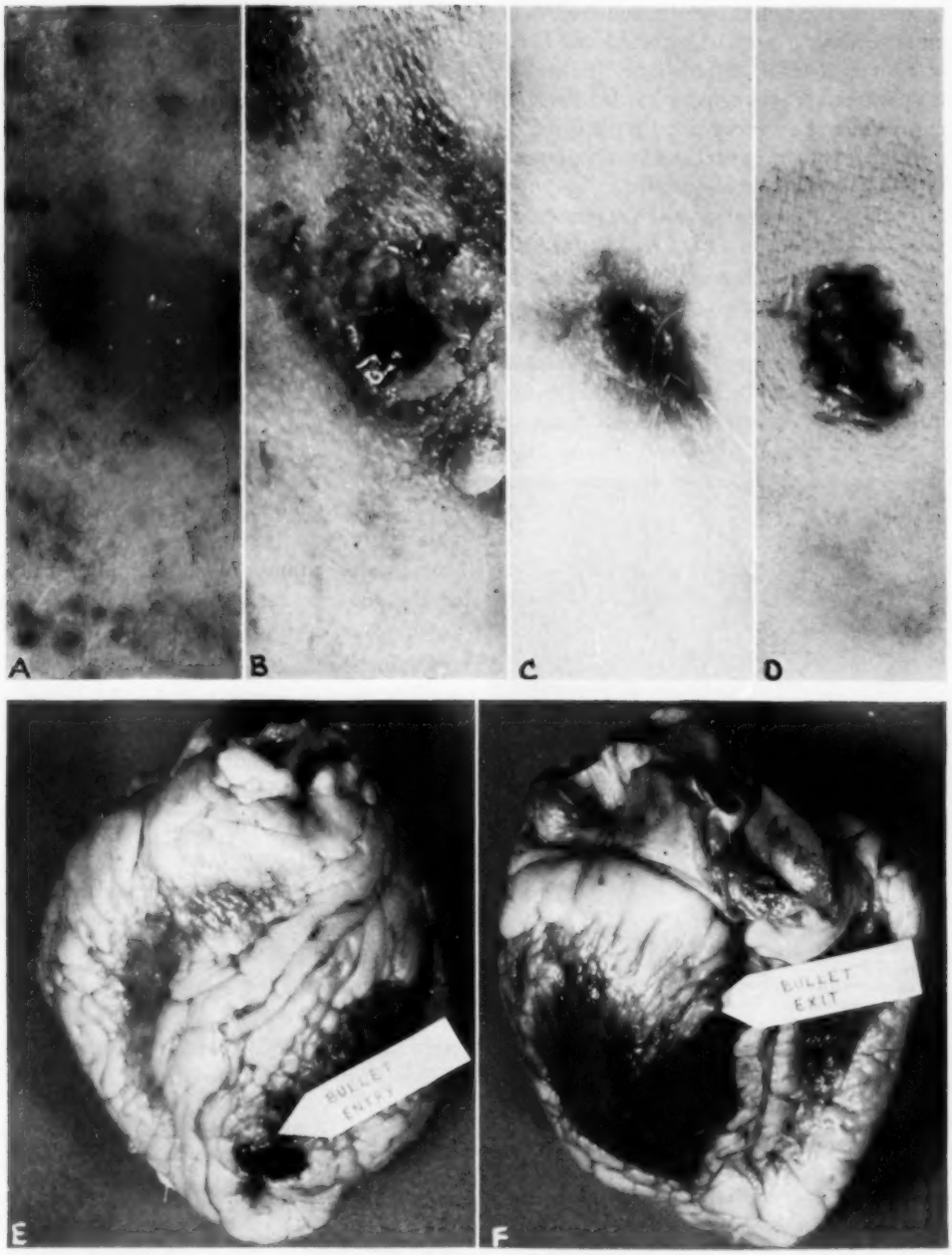


Fig. 5.—Appearance of entrance and exit points of bullets.

*A*, entrance of 22-caliber bullet with muzzle held horizontally at a distance of 6 in. from the target. Note the circumferential hemorrhage, burns, and halo of powder granules. *B*, exit wound of bullet in *A*. *C*, entrance of a 22-caliber bullet with the muzzle held at an angle of 45° at a distance of 11 ft. *D*, exit wound of bullet in *C*. Note the difference in shape of hole in *B* and *D* due to angle of entrance of bullet. Unless the missile is deflected by bone, the shapes of entrance and exit holes tend to approximate. *E*, heart, showing entrance wound. *F*, the exit wound of *E*. Note the tearing effect of the missile leaving the organ.

tissues. If the bullet had encountered bones on its course, it may be flattened and buried in torn muscle or within bone. If the bone is splintered into fragments, it becomes still more difficult to recognize by palpation the difference between metal and bone fragments. A photograph of holes produced by bullets of the more commonly used calibers can be kept for comparison (Fig. 4*C* and *D*).

Exit wounds are usually larger than entrance wounds, except for contact type or because of peculiar conditions of angle of firing, interfering objects, and deflections. An entrance wound inflicted by a bullet fired from a short distance (Fig. 5*A*) may appear,

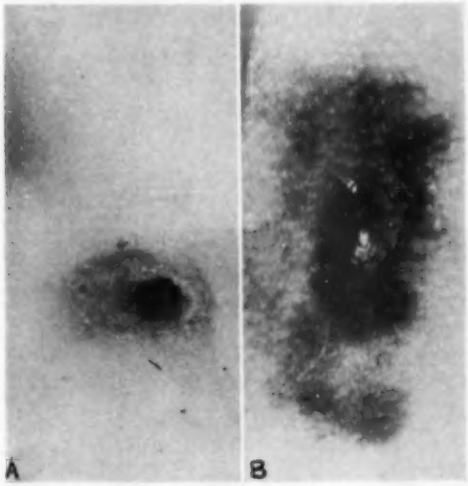


Fig. 6.—Differentiation of antemortem and postmortem wounds.

*A*, postmortem wound produced by a 22-caliber bullet discharged 9 ft. from the target. Note absence of hemorrhage in the edges and the neat perforation. Contrast it with *B*, a wound produced also by a 22-caliber bullet discharged at a similar distance of 9 ft. Note the circumferential hemorrhage and the ragged perforation.

or even be, larger than the exit (Fig. 5*C*) because of tissue destruction. Exit wounds made by bullets of the same caliber but fired at different distances may vary in size (Fig. 5*C* and *D*). Organs present less difficulties in differentiation. The entrance wound of the heart is usually small in comparison with the very large, ragged exit hole (Fig. 5*E* and *F*).

It is sometimes essential to differentiate antemortem from postmortem wounds. One of the significant differences is the absence after death of hemorrhage into the edges of the wound (Fig. 6*A* and *B*) and in the deeper tissue through which the missile passes. When the target is a long distance away from the muzzle, hemorrhage may be at a minimum but is present. Postmortem wounds may show brown fluid or brown discoloration due to hemoglobin pigments. The wound produced after death shows little or no tearing of tissue and, hence, is apt to show a rather neat perforation.

## SUMMARY

Gunshot wounds present to the examining physicians several changes which make possible interpretations of distance and direction of the muzzle, caliber of the missile, and antemortem or postmortem infliction.

Evaluation and interpretation of gunshot wounds require certain technical information of firearms and knowledge of morphological changes of tissue and materials induced by missiles under various conditions.

The examining physician should be familiar with the character of information which law-enforcing agencies require of him. He should be aware also of the limitations inherent in his work.

The examining physician should be preferably a pathologist. A complete and thorough autopsy is essential in every death presumably due to a gunshot wound.

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# Fatal Fulminant Acute Carbon Tetrachloride Poisoning

ROBERT B. JENNINGS, M.D., Chicago

Carbon tetrachloride, $CCl_4$, is an inexpensive noninflammable volatile solvent which is used extensively in industry and in the home. Many cases of poisoning due to inhalation or ingestion of this compound have been reported in the literature of the last several decades,* and there has been much interest in the pathogenesis of its toxic action. Eight cases of fatal carbon tetrachloride poisoning are reported in this paper. For ease in presentation, they have been divided into three groups, each of which is characterized by a different clinicopathologic picture: a renal failure group, an anesthetic group, and a fulminant group.

Most cases of fatal $CCl_4$ poisoning fall into the renal failure group. The patients usually die 9 to 12 days after exposure and are oliguric or anuric at some time during their illness. Postmortem examination usually reveals centrilobular necrosis in the liver, lower nephron nephrosis in the kidney, and varying degrees of pulmonary edema or pneumonitis. Seventy-one of the 75 cases of fatal $CCl_4$ poisoning on file at the Armed Forces Institute of Pathology (A. F. I. P.) fall into this category. Three such cases are included in this paper.

A small number of patients die from anesthetic effects, either during exposure or several hours thereafter. These patients have signs of medullary depression and die before characteristic pathologic lesions have developed. One such case is included in this report.

A new group of patients dying after a fulminant course and with characteristic clinical and pathologic findings is the subject of this paper. The group consists of four patients who died rapidly, four days or less after exposure to $CCl_4$, and without showing remarkable signs of renal failure. These patients all had similar pathologic findings, the most striking of which were in the liver and kidney. The liver was the seat of massive zonal necrosis and fat infiltration, which involved all cells of the lobule except for scattered parenchymal cells in the periportal region. The cells of the proximal tubules of the kidney contained much subnuclear neutral fat. These cases have been designated as fulminant acute $CCl_4$ poisoning because of the rapid clinical course and unusually extensive liver disease.

## MATERIALS AND METHODS

Clinical summaries, autopsy reports, and microscopic sections of liver and kidney were obtained on Cases 1, 3, 4, 5, and 6 from the Armed Forces Institute of Pathology. No wet tissue was available on these cases. Wet tissue, microscopic sections, clinical history, and autopsy protocols were available on Cases 2,† 7,‡ and 8.‡ Fat stains[66]; periodic acid-Schiff,[67] Wilder's reticulum,[68] and iron stain,[69] and Ralph's hemoglobin stain[70] were applied to sections of liver and kidney of the last three cases.

Aided by a grant from the Chicago Heart Association.

From the Department of Pathology, Northwestern University Medical School.

Presented at the Fifty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, Philadelphia, April 17, 1954.

This study was made possible through the courtesy of the Armed Forces Institute of Pathology. Five of the eight cases reported are from the files of the Institute.

* References 1 through 65.

† From Passavant Memorial Hospital, with the permission of Dr. A. J. Atkinson.

‡ From U. S. N. H. 13, Great Lakes, Ill.

*Clinical Findings.*—The pertinent findings in the case histories are listed in Table 1, and a brief summary of each case is given below. The patients were men, and all except one were in the military service. Their ages varied from 21 to 45 years. The alcoholic intake of all but one of the patients, on whom this information was not available, was classified as moderate to heavy. The patients lived 2 to 312 hours after exposure to $CCl_4$; four ingested the material and four inhaled it. Jaundice developed in the five patients who lived 60 hours or more.

## SUMMARY OF EIGHT CASES

Case 1.—P. W. J. was a 31-year-old white male Army Sergeant, A. F. I. P. No. 269373.

Kidneys: The kidneys were grossly normal and weighed 355 gm.

Liver: The liver weighed 2100 gm., but otherwise it appeared to be normal.

Lungs: The right and left lungs weighed 1125 and 725 gm., respectively, and were markedly edematous.

Case 2.—W. C. B. was a 45-year-old white male salesman, P. M. H. A-1716.

*Clinical History.*—The history was obtained from the patient's wife. About 48 hours prior to admission to Passavant Memorial Hospital, he spent several hours in a closed room cleaning furniture with $CCl_4$. Two dogs were in the room at the same time, and one died six to eight hours later and the other, the next morning. Thirty-six hours before admission, the patient began vomiting. The vomiting became worse, and he complained of pain in the right upper quadrant and epigastrium. On admission he was incoherent and unable to give a history. He had had intermittent diarrhea and gnawing midabdominal pains, relieved by foods, as well as fat intolerance for a long time. Gall bladder and gastrointestinal x-rays, liver-function tests, and stool examinations made one year previously were normal. He was an occasional drinker. Physical examination on admission showed a moderately cyanotic man who writhed in bed, moaned incoherently, and had gasping respirations. The blood pressure was 60/40 and soon became unobtainable.

Table 1.—*Clinical Summaries in Tabular Form*

| Case | Route of $CCl_4$ | Alcohol | Survival, in Hours | Jaundice * | Renal Failure | Renal Lesion | Hepatic Lesion |
|---|---|---|---|---|---|---|---|
| Case 1 Age 38 AFIP 269373 | Inhalation | Drinking at time of exposure | 2 | Absent | Absent | Normal | Normal |
| Case 2 Age 45 A-1716 | Inhalation | Occasional drinker | 51 | Absent | NPN 90 mg./100 cc. | Fat nephrosis | Confluent zonal necrosis |
| Case 3 Age 37 AFIP 84759 | Ingestion | Drinking at time of exposure | 58 | Absent | NPN 70 mg./100 cc. | Fat nephrosis | Confluent zonal necrosis |
| Case 4 Age 21 AFIP 104604 | Ingestion | Not recorded | 60 | I. I., 40 | NPN 128 mg./100 cc. | Fat nephrosis | Confluent zonal necrosis |
| Case 5 Age 22 AFIP 79867 | Ingestion | Moderate drinker | 96 | I. I., 120 | Oliguric | Minimal fat nephrosis | Centrilobular and midzonal necrosis |
| Case 6 Age 35 AFIP 150578 | Ingestion | Heavy drinker | 116 | Present | Anuric | Lower nephron nephrosis | Centrilobular necrosis |
| Case 7 Age 25 USNH 271-49 | Inhalation | Heavy drinker | 240 | Bilirubin 15 mg./100 cc. | Oliguric NPN 136 mg./100 cc. | Lower nephron nephrosis | Centrilobular necrosis |
| Case 8 Age 38 USNH 121-49 | Inhalation | Drinking at time of exposure | 312 | Present | Anuric NPN 160 mg./100 cc. | Lower nephron nephrosis | Centrilobular necrosis |

* I. I., icterus index.

*Clinical History.*—This patient was found dead in bed at 10 p. m., March 11, 1945. A small cup filled with $CCl_4$ and containing a rag was found wedged between the mattresses of his bed. The patient had been alone in the bedroom for two hours before he was found. He was considered to be a moderate drinker.

*Laboratory Data.*—Postmorten blood contained no alcohol.

*Gross Autopsy Findings.*—Brain: The brain weighed 1700 gm. and was grossly and microscopically normal. Toxicologic analysis of the brain revealed 635.6 mg. of $CCl_4$ per kilogram of wet tissue.

The heart sounds were faint, and the lungs were clear. The abdomen was tender in the right upper quadrant and rigid. The patient died 3 hours after admission to the hospital, 51 hours after exposure to $CCl_4$.

*Laboratory Data.* — Premortem Blood: RBC 2,880,000, Hb 10.5 gm. per 100 cc., WBC 6000. Serum amylase 310 units.

Postmortem Blood: Blood failed to clot. Van den Bergh—direct: 1 minute 0.98 mg. per 100 cc., 30 minutes 1.38 mg. per 100 cc.; indirect: 2.07 mg. per 100 cc. BUN 19.3 mg. per 100 cc.; NPN 90 mg. per 100 cc.; creatinine 5.71 mg. per 100 cc., and thymol turbidity 7.3 units.

*Gross Autopsy Findings.*—Kidneys: The kidneys were grossly normal and weighed 250 gm.

Liver: The liver weighed 2050 gm. The surface was smooth and brown. On section, the surface was alternating red and yellow, and architectural details were difficult to identify.

Lungs: The lungs were grossly normal and weighed together 975 gm.

CASE 3.—A. W. C. was a 37-year-old white male Army Private, A. F. I. P. No. 84759.

*Clinical History.*—The patient walked into the Station Hospital, Camp Haan, Calif., at 4:30 p. m., Aug. 19, 1942, with a chief complaint of vomiting for two days. During the night after admission he complained of abdominal pain, and this was relieved by 0.25 gm. of morphine sulfate. At 5 a. m. he walked to the bathroom, was unable to void, and was seen to be weak and trembling. Caffeine and sodium benzoate was given intravenously. The pulse remained weak, and the patient was unable to respond to questions. He died at 9:15 a. m., having been given oxygen and epinephrine. Further investigation revealed that he had ingested an unknown quantity of carbon tetrachloride over the period of 48 to 72 hours before death. He had been drinking alcohol heavily prior to taking the carbon tetrachloride and was apparently attempting to sober-up with this compound.

*Laboratory Data.*—Blood sugar: 84 mg. per 100 cc., NPN 70 mg. per 100 cc.

Urine: Albumin 2 +, sugar negative, microscopic findings negative.

*Gross Autopsy Findings.*—Kidneys: The kidneys were grossly normal and weighed together 390 gm.

Liver: The liver weighed 1690 gm. and had a mottled-tan external and cut surface.

Lungs: The right and left lungs weighed 620 and 660 gm., respectively, and were grossly normal.

CASE 4.—D. T. was a 21-year-old white Army Private, A. F. I. P. No. 104604.

*Clinical History.*—The patient was admitted to a dispensary on July 5, 1942, with a history of having accidentally taken "a small drink" of $CCl_4$ (fire extinguisher fluid) 15 minutes before admission. The patient said he spit it out immediately and vomited. He was given emetics and large quantities of water to drink during the next one and one-half hours, continued to be nauseated, and vomited repeatedly during the remainder of the day. The patient was transferred to the Fifth General Hospital on the morning of July 6. Physical examination revealed a pale definitely icteric white man, who was repeatedly vomiting small amounts of blood. The abdomen was not tender, and the liver was not palpable. For a short time after admission he continued to vomit dark-brown bloody watery fluid. He was given 1000 cc. of 2.5% dextrose in isotonic saline intravenously. At 7 p. m. the blood pressure was 80/0. The lungs remained clear. At 10:30 p. m., 20 cc. of 10% calcium gluconate was given intramuscularly, and an hour later a subcutaneous infusion of 1000 cc. of 2.5% dextrose in saline was given. At 2 a. m. on July 7, the blood pressure was 60/20 and the pulse rate was 132. He was mildly delirious. He was given 5 cat units of Digifolin intravenously. At 2 p. m. the patient became cyanotic; the respirations were feeble and irregular, and the blood pressure was 45/0. Oxygen was administered, and the patient died quietly 60 hours after ingestion of the carbon tetrachloride.

*Laboratory Data.*—July 6, 1942: RBC 3,900,000, Hb 87%, WBC 23,000, icterus index 40 units. July 7: Hb 73%, NPN 128 mg. per 100 cc. Urinalysis showed 1+ albumin, bile negative, microscopic findings showed 0-20 WBC/h. p. f. (centrifuged).

*Gross Autopsy Findings.*—Kidneys: The kidneys weighed 420 gm. The cut surface bulged.

Liver: The liver weighed 1450 gm. The cut surface was pale yellow with small purplish-red areas marking the central zones.

Lungs: The lungs were grossly normal. No weights were recorded.

CASE 5.—F. S. was a 22-year-old Puerto Rican Private, A. F. I. P. No. 79867.

*Clinical History.*—F. S. was admitted to a station hospital with complaints of vague abdominal pains, loss of weight, and weakness. A gastrointestinal series was negative. Gastric analysis was normal. Stool examination was positive for Necator americanus. Four capsules containing a total of 5.3 cc. of $CCl_4$ and 2.6 cc. of oil of Chenopodium were administered on Dec. 31, 1941. The patient was noted to have marked nausea and vomiting throughout Jan. 2 and 3, 1942, and this was treated with 1500 cc. of dextrose in isotonic saline. During the evening of Jan. 3, the patient was restless and uncooperative, and late in the evening he went into a coma. He was treated with dextrose, morphine, and atropine, but he remained comatose. Icterus was noted before death, as were rales in the lungs. Five

hundred cubic centimeters of 10% dextrose in isotonic saline was administered during the morning of Jan. 4, and he died at 11:25 a. m.

*Laboratory Data.*—Jan. 4, 1942: Icterus index 120 units; blood sugar, 96 mg. per 100 cc.

*Gross Autopsy Findings.*—Kidneys: The kidneys weighed 450 gm. and were grossly normal.

Liver: The liver weighed 1360 gm. The external surface was red and yellow, and the cut surface contained irregular small pink areas alternating with mahogany-brown parenchymal tissue.

Lungs: A large amount of edema was noted, as was passive hyperemia in lower lobes. No weights were recorded.

CASE 6.—W. J. C. was a 35-year-old white male T/5, A. F. I. P. No. 150578.

*Clinical History.*—W. J. C. was admitted to the hospital at 10:30 p. m. on Aug. 29, 1945, with a history of having swallowed about a tablespoonful of $CCl_4$ by mistake. He drank water immediately, and within 15 minutes was given an emetic and vomited. The man was a heavy drinker but denied having anything to drink on the day of his admission. The patient was sluggish in his movements and speech, but his sensorium was clear and cooperation was good. On Aug. 31, nausea and vomiting were noted throughout the day and an infusion of 5% dextrose in saline was given. The vomitus contained food and was blood-stained. The patient vomited repeatedly on Sept. 1. Intravenous 10% dextrose, calcium, and thiamine were given. Oliguria was noted on Sept. 2. Nausea and vomiting continued. Increasing oliguria and slight edema were noted on Sept. 3. The patient developed rapid deep respirations and a fall in blood pressure during the afternoon and died quietly at 8 p. m. The urinary output on Sept. 3 was 30 cc.

*Laboratory Data.*—Sept. 2, 1945: RBC 3,100,000. Twenty-four-hour urine output, 230 cc. with 3+ albumin, bile, granular casts, leucine crystals.

*Gross Autopsy Findings.*—Kidney: The kidneys weighed together 440 gm. There were occasional subcapsular petechiae.

Liver: The liver weighed 1750 gm. The capsule was smooth, and the underlying parenchyma, golden pale-yellow. The lobular pattern was not distinct on the cut surface. The organ was friable.

Lungs: The right lung weighed 860 gm., and the left, 970 gm. The trachea and bronchi were filled with blood-tinged fluid. The cut surfaces were wet.

CASE 7.—L. C. A. was a 25-year-old white male Navy AO2, U. S. N. H. No. 13, No. 121-49.

*Clinical History.*—L. C. A. had been on a spree the evening of Feb. 1, 1949, and had cleaned his blue uniform in a bucket of $CCl_4$ on the afternoon of Feb. 3. He had consumed more alcohol while wearing his uniform, which was still damp with $CCl_4$, on the evening of Feb. 3. Beginning about 11 p. m., he became nauseated and started to vomit. He was admitted to a dispensary on the following morning in a semiconfused state, still vomiting.

He was given supportive therapy, including 3000 cc. of intravenous dextrose, containing calcium lactate. He stopped vomiting in the morning of Feb. 5 and began to complain of epigastric pain. He was transferred to U. S. N. H. No. 13 at 5:45 p. m. on Feb. 5. On admission he was semistuporous, and his liver was tender and palpable 2.5 cm. below the right costal margin. Admission laboratory work revealed a WBC of 15,450, hemoglobin 14 gm. per 100 cc., and 1+ albuminuria. He was treated with methionine, menadione sodium bisulfite (Hykinone), calcium gluconate, and intravenous dextrose. Intermittent vomiting was noted during the evening of Feb. 5 and in the morning of Feb. 6. His urinary output consisted of 50 cc.

TABLE 2.—*Summary of Laboratory Data for Case 7*

| 24-Hr. Period Ending (Date) | 24-Hr. Fluid Intake (I. V.), Cc. | 24-Hr. Fluid Output | | $CO_2$, Vol. % | Cl, Mg./100 Cc. as NaCl | NPN, Mg./100 Cc. |
|---|---|---|---|---|---|---|
| | | Emesis | Urine | | | |
| 2/ 7 | .... | .. | ... | .. | ... | 86 |
| 2/ 8 | 2050 | .. | 285 | 52 | 260 | 108 |
| 2/ 9 | 2530 | .. | 100 | .. | ... | .. |
| 2/10 | 1060 | .. | 115 | .. | ... | .. |
| 2/11 | 1200 | 35 | 160 | 52 | 420 | 136 |
| 2/12 | 150 | 119 | 195 | 43 | ... | .. |
| 2/13* | 690 | 330 | 150 | 42 | 320 | .. |
| Total | 7080 | 484 | 1005 | | | |

* Ending at 8 a. m.

of dark urine with a specific gravity of 1.020, which contained a trace of albumin and had 5-10 RBC/h.p.f. His 24-hour urinary output varied from 100 to 285 cc. between Feb. 7 and Feb. 13. The specific gravities varied from 1.010 to 1.020, and the urines consistently contained 2 to 4+ albumin. The NPN rose gradually, reaching 136 mg. per 100 cc. on Feb. 11. The jaundice deepened, the serum bilirubin reaching 15 mg. per 100 cc. on Feb. 12. The blood pressure increased, rising from 138/70 on Feb. 9 to 168/98 on Feb. 11. Increasing dyspnea, rales in the chest, rapid pulse rate, and other signs of pulmonary edema became more and more prominent. Intravenous digitalis was given on Feb. 12, and oxygen was given by tent. The patient died in respiratory distress at 10 p. m., on Feb. 13.

*Laboratory Data.*—Summarized in Table 2.

*Gross Autopsy Findings.*—Kidneys: The kidneys weighed together 520 gm. The cortex was pale, and the medulla, congested.

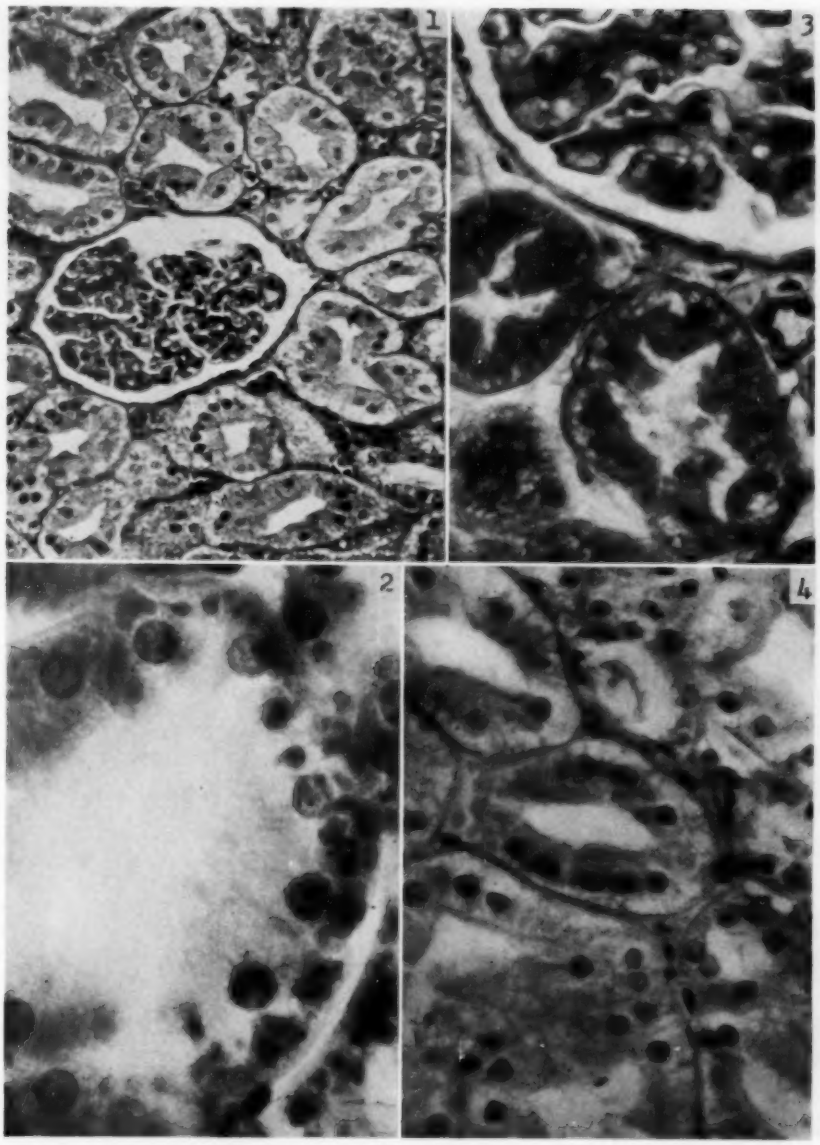



Fig. 1 (Case 4).—Section of kidney. Note the vacuolated basal portion of the proximal tubular cells. Sudan III stain showed the vacuoles to contain neutral fat. The glomerulus is microscopically normal. Hematoxylin and eosin; × 150.

Fig. 2 (Case 2).—Frozen section of a proximal tubule. Note the fat at the base of the proximal tubular cells. Sudan III stain, green filter; × 720.

Fig. 3 (Case 5).—Section of kidney. Note the vacuolation of the basal portion of the proximal tubular cells and the intact basement membrane. Masson stain; × 216.

Fig. 4 (Case 3).—Section of kidney. Two vacuole-free distal tubules are present at one and four o'clock. Hematoxylin and eosin; × 360.

Liver: The liver weighed 1600 gm. The cut surface showed small red and brown areas, between which there were large zones of yellow tissue.

Lungs: The lungs weighed together 1900 gm. The cut surface had a deep grayish-purple mottled appearance and exuded much edema fluid, and the

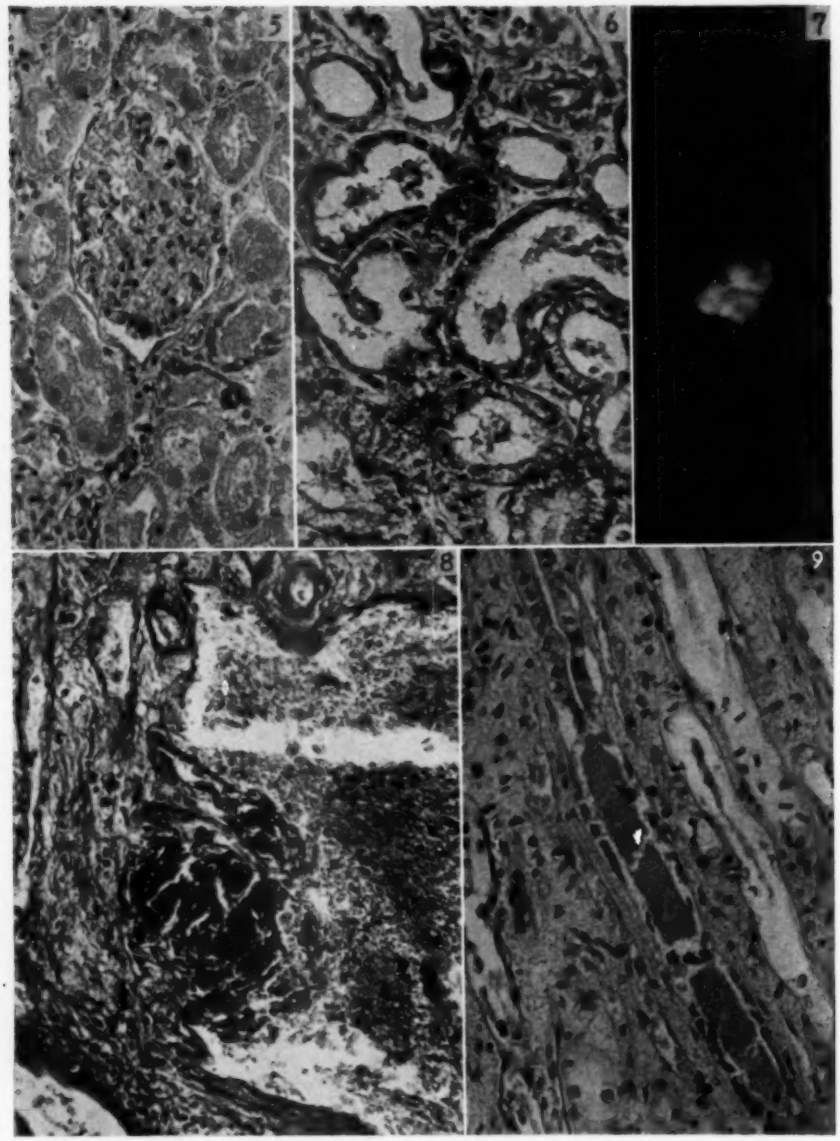



Fig. 5 (Case 6).—Section of kidney. The cytoplasm of the proximal tubular cells is free of vacuoles, though some cloudy swelling is present. A calcosphere is present at four o'clock. Hematoxylin and eosin; × 180.

Fig. 6 (Case 8).—Section of kidney. Note the extensive cloudy swelling and hydropic degeneration of the proximal tubular cells. Sudan III stains were negative for neutral fat. Note the calcosphere at 12 o'clock. Hematoxylin and eosin; × 180.

Fig. 7 (Case 8).—Photomicrograph taken under crossed Nicol prisms of a calcosphere in a kidney section, showing the anisotropic character of this structure; × 200.

Fig. 8 (Case 7).—Section of kidney showing a tubulovenous thrombus in a large vein. Periodic acid-Schiff stain; × 180.

Fig. 9 (Case 3).—Section of kidney, showing a cast in a collecting duct. Note the flattened and partially necrotic epithelium around the cast. Hematoxylin and eosin; × 180.

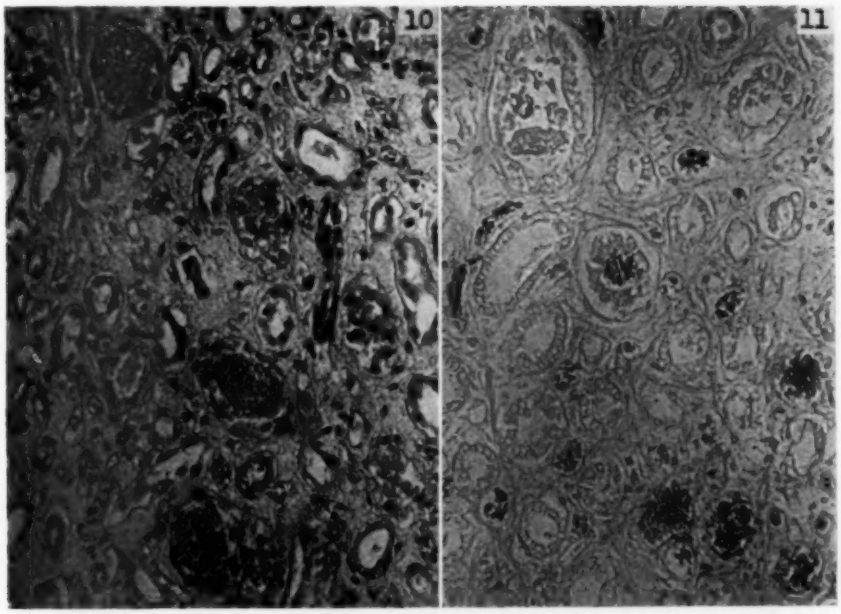



Fig. 10 (Case 6).—Section of renal medulla, showing four dilated collecting ducts filled with granular golden-brown casts (so-called heme casts). Hematoxylin and eosin; × 180.

Fig. 11 (Case 7).—Section of renal medulla stained for hemoglobin by Ralph's technique (no counterstain used), showing faintly hemoglobin-positive casts in the collecting ducts.

tracheobronchial tree was filled with frothy hemorrhagic material.

CASE 8.—H. G. G. was a 38-year-old white male Army Sergeant, U. S. N. H. No. 13, Autopsy No. 271-49.

*Clinical History.*—The patient was admitted to a station hospital on March 24, 1949, complaining of headache, nausea and vomiting, and epigastric pain of two days' duration. On March 20, he cleaned a small rug with $CCl_4$ in the open air near his trailer home. He got his hands fairly wet with $CCl_4$, inhaled some of the fumes, and put the rug, still wet, in the trailer that night. Further history revealed that he was a heavy drinker. At the station hospital, despite adequate intravenous fluids, spinal anesthesia, intravenous sodium sulfate, and ureteral catheterization, he remained practically anuric and was transferred to U. S. N. H. No. 13 on March 28. The patient remained oliguric despite all efforts at treatment, which consisted of glucose in water in limited amounts, intravenous sodium lactate, oxygen, and digitalis. His NPN was 92.5 mg. per 100 cc. and rose steadily to 160 mg. per 100 cc. the day before death. His blood pressure was 160/110 on admission and remained high until his condition became terminal. Dyspnea, hemoptysis, rales in the chest, and disorientation were the principal findings in the last three days of his hospital course. He died quietly at 10:28 p. m., on April 2.

TABLE 3.—*Summary of Laboratory Data for Case 8*

| 24-Hr. Period Ending (Date) | 24-Hr. Fluid Intake (I. V.), Cc. | 24-Hr. Fluid Output, Urine | $CO_2$, Vol. % | Cl, Mg./100 Cc. as NaCl | NPN, Mg./100 Cc. |
|---|---|---|---|---|---|
| 3/25 | 3000 | 0 | .. | .. | .... |
| 3/26 | 1000 | 0 | .. | .. | .... |
| 3/27 | 2250 | 0 | .. | .. | .... |
| 3/28 | 800 | 0 | 30 | 400 | 92.5 |
| 3/29 | 850 | 90 | 36 | 350 | 95.5 |
| 3/30 | .... | 183 | .. | .. | .... |
| 3/31 | 950 | 88 | .. | .. | .... |
| 4/ 1 | 1500 | 75 | .. | .. | 160 |
| 4/ 2 | 1500 | .. | .. | .. | .... |
| Total | 11,850 | 436 | | | |

*Laboratory Data.*—Summarized in Table 3.

*Gross Autopsy Findings:*

Kidneys: The kidneys weighed 560 gm. The capsules were tense and stripped with ease.

Liver: The liver weighed 2300 gm., and the cut surface was mottled red and yellow.

Lungs: The right lung weighed 1700 gm., and the left, 1350 gm. Consolidation was noted in the right upper lobe, and there were focal consolidated areas throughout the remainder of the lung.

## HISTOPATHOLOGY

The most striking findings in the fulminant cases were limited to the liver and kidney.

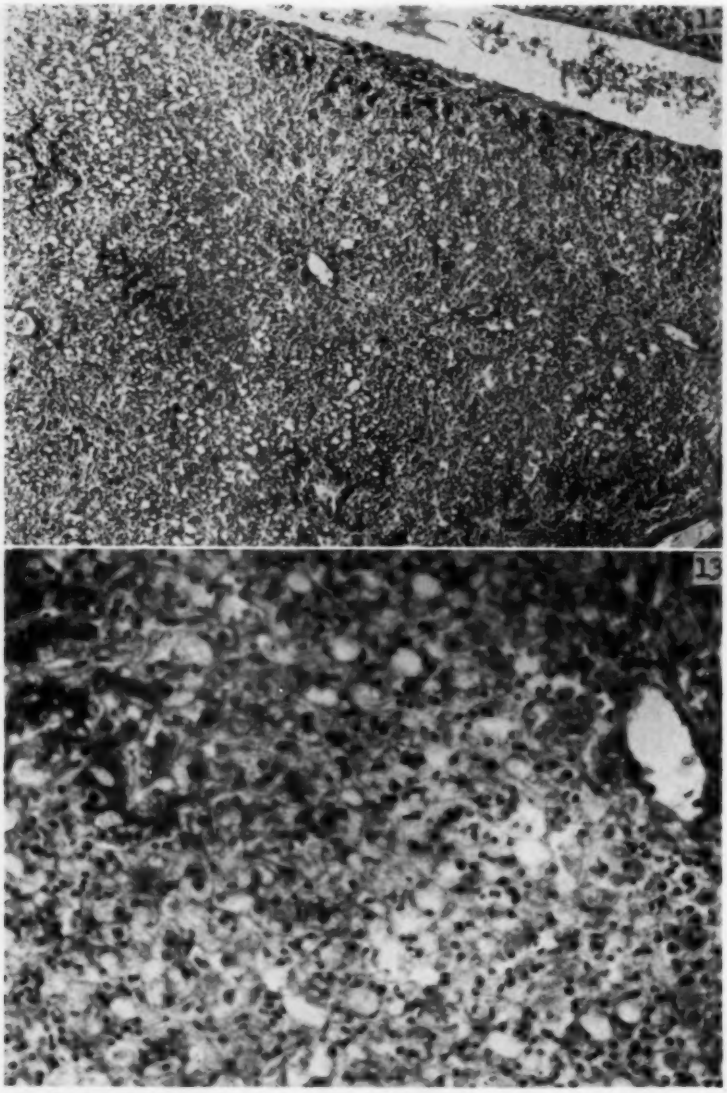


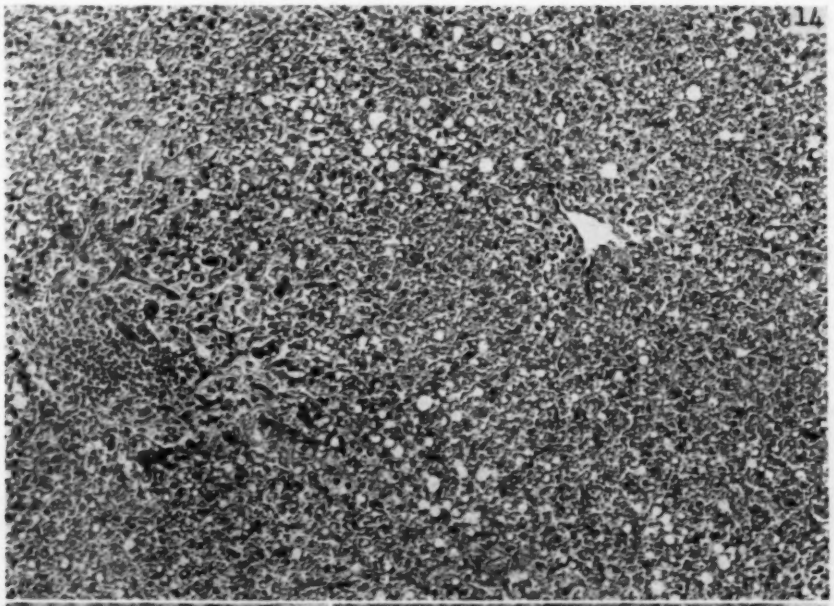
Fig. 12 (Case 4).—Section of liver, showing the massive character of the hepatic necrosis. Hematoxylin and eosin; reduced ⅔ from mag. × 36.

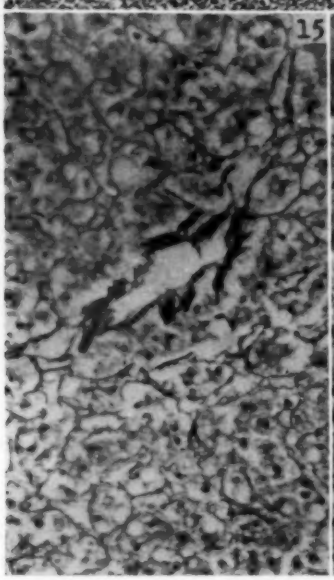
Fig. 13 (Case 4).—High-power view of the central vein shown in the middle of Figure 12. Note the vacuolation of the cytoplasm of the few remaining hepatic cells which are visible on the left side of the photomicrograph. Reduced ⅔ from mag. × 180.

Pulmonary edema and, frequently, bronchopneumonia were noted, in addition to changes in the liver and kidney in the patients who followed the usual course of fatal $CCl_4$ poisoning. Remarkable findings were not noted in other organs.

*Kidney.*—No gross or microscopic abnormalities were noted in the kidneys of the first patient, who died during exposure to $CCl_4$ fumes and probably represents an anesthetic death.

Cases 2, 3, and 4 are typical of the fulminant group. The kidneys were slightly enlarged, and the parenchyma bulged on cut section. Microscopic examination revealed histologically normal glomeruli except for oc-



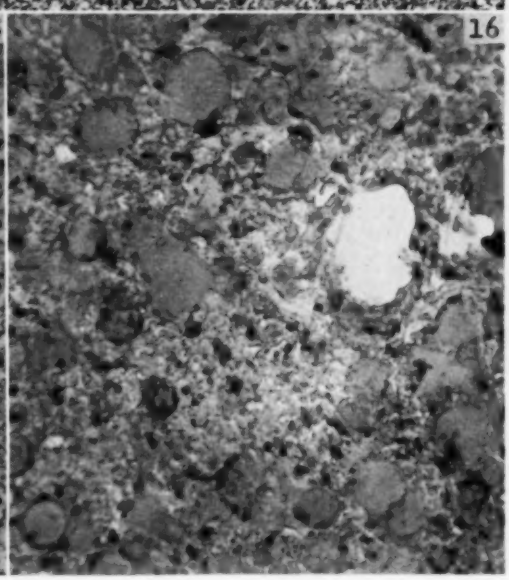




Fig. 14 (Case 3).—Low-power photomicrograph of section of liver, showing a portal triad on the left side of the figure surrounded by a small number of intact parenchymal cells. Hematoxylin and eosin; × 65.

Fig. 15 (Case 3).—Liver showing a central vein and open reticulum around it. Wilder's reticulum stain; × 216.

Fig. 16 (Case 2).—Frozen section of liver, showing a central vein surrounded by neutral fat containing parenchymal cell ghosts. Note the massive nature of this fatty change. Sudan III stain; × 360.

casional small droplets of neutral fat. The proximal tubules contained a large amount of Sudanophilic material, which occupied a basal position in the cell, between the nucleus and basement membrane. This neutral fat was in large globules (Figs. 1 to 4). Only

a small number of fine droplets of supranuclear fatty material were present. The ascending limbs of Henle's loops also contained large amounts of basal fat. The distal convoluted tubule and collecting ducts contained occasional droplets. Periodic acid-Schiff, hemoglobin, and iron stains in Case 2 showed no abnormalities. The tubular basement membranes appeared normal with Wilder's reticulum stain.

Case 5 falls into the fulminant group, although the changes are not marked, for only small numbers of small droplets of basal fat were present (Fig. 3). Occasional heme casts were present in the collecting ducts. Another of the fulminant cases, Patient 3, also had some granular casts in the collecting ducts (Fig. 9).

Cases 6, 7, and 8, where the survival time exceeded 120 hours, demonstrated the usual or expected renal findings in $CCl_4$ poisoning. The kidneys were enlarged, weighing together 430, 560, and 520 gm., respectively. The glomeruli were histologically normal, though a large amount of protein precipitate material was present in Bowman's space. The proximal tubules were not frequently dilated, and the tubular epithelial cells were swollen and the cytoplasm granular (Figs. 5 and 6). Occasional small droplets of intracytoplasmic supranuclear Sudanophilic material were noted. Calcospheres were present in the lumens of the proximal tubules (Figs. 5 and 6) in all three of these cases. These bodies were anisotropic (Fig. 7) and basophilic, and lost their anisotropic properties shortly after exposure to periodic acid. The ascending limbs of Henle's loops, distal convoluted tubules, and collecting ducts contained numerous heme and granular casts (Fig. 10). These casts took a faint hemoglobin stain, using the benzidine-hydrogen peroxide technique (Fig. 11). The epithelium was flattened around the casts, and occasional tubular cells were necrotic. The greatest percentage of casts were present in the medulla. Many granulomatous foci around casts were noted in the cortex, as were occasional tubulovenous communications with thrombosed veins (Fig. 8).

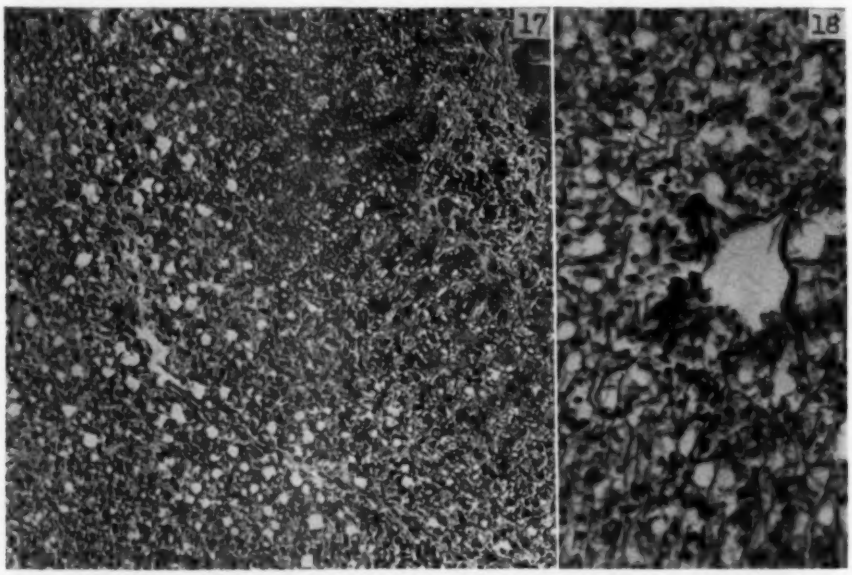



Fig. 17 (Case 5).—Section of liver, showing a rim of intact parenchymal cells surrounding a portal triad on the right side of the figure. Hematoxylin and eosin; × 65.

Fig. 18 (Case 5).—Section of liver, showing a central vein surrounded by intact reticulum. Wilder's reticulum stain; × 216.

*Liver.*—Centrilobular necrosis and fat accumulation was noted in all patients except

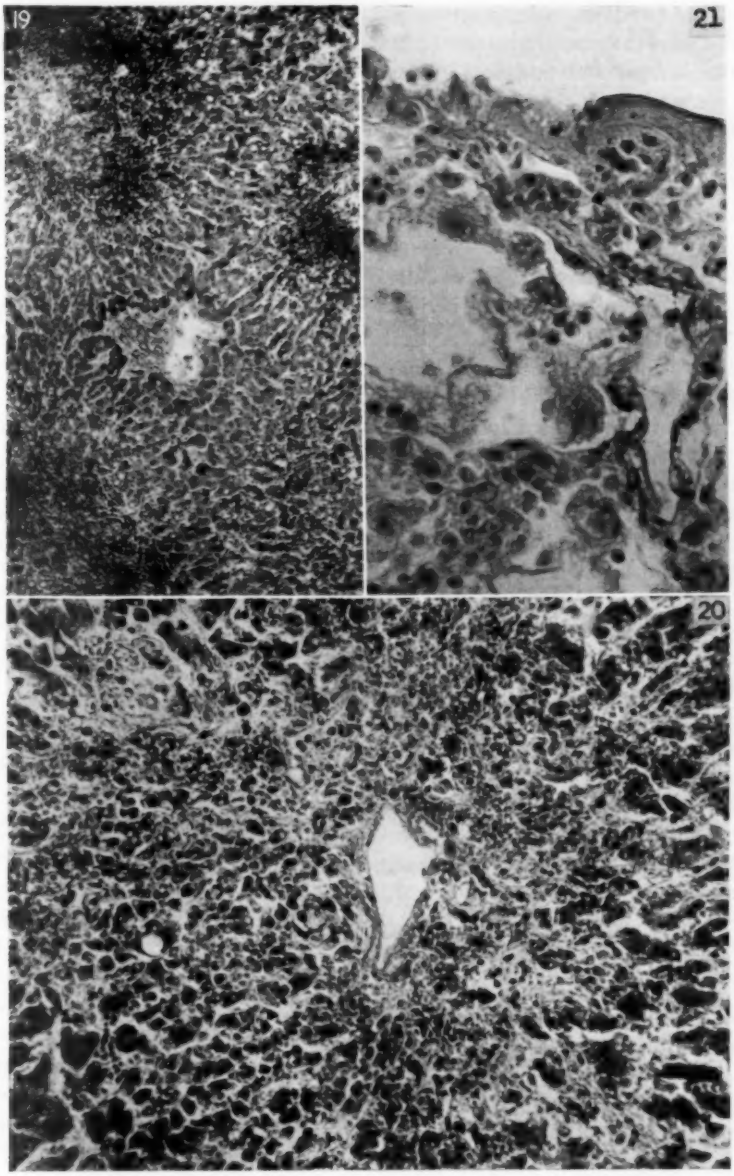



Fig. 19 (Case 8).—Section of liver. Note the absence of parenchymal cells and marked passive hyperemia in central portion of lobule. Hematoxylin and eosin; × 70.

Fig. 20 (Case 8).—Higher power view of liver shown in Figure 19. Note marked passive hyperemia and incomplete parenchymal cell regeneration; × 180.

Fig. 21 (Case 8).—Section of lung. Note the homogeneous hyaline membrane at the top of the figure and the epithelial lining of the alveolus in the middle of the figure. Hematoxylin and eosin; × 216.

Case 1, where the only finding was congestion. Jaundice was noted in Patients 4 to 8, all of whom lived 60 hours or longer.

The liver necrosis in the patients with a fulminant course, Cases 2, 3, and 4, was massive (Figs. 12, 13, 14, 16); small islands of

periportal vacuolated fat containing parenchymal cells were all that remained of the lobules. The central and midzonal regions contained necrotic cells, much neutral fat (Fig. 16), and areas of hemorrhage. The reticulum network was collapsed in some areas but in general was held open by fat-filled parenchymal cell ghosts (Figs. 15 and 18). The microscopic picture in Patient 5 was similar, though the necrosis was less massive (Fig. 17) and more intact periportal parenchymal cells were visible.

Patients 5, 7, and 8, who all had varying degrees of renal failure, differed from the fulminant cases in that the hepatic lesion was much less marked. Patient 6, who died five days after exposure, had the most extensive lesion in this group. The entire central portion of the lobule was filled with necrotic neutral fat-laden parenchymal cells. Patients 7 and 8 survived 10 and 13 days, respectively, and the area where the necrosis had occurred contained numerous lipid- and, occasionally, hemosiderin-containing macrophages (Figs. 19 and 20). The reticulum network was still open, and regenerative changes were noted in the surrounding parenchymal cells. The histologic picture in the last two cases was complicated by the presence of a marked degree of passive hyperemia (Figs. 19 and 21).

*Lung.*—Massive pulmonary edema was present in the last three patients, who all survived for 116 hours or more. Bronchopneumonia of variable degree was also present, as were the findings of so-called uremic pneumonitis (Fig. 21). Marked pulmonary edema was also present in Case 1. Minimal amounts of pulmonary edema were noted in Cases 2 to 6.

## COMMENT

Most patients who die or develop symptoms of $CCl_4$ poisoning fall into the renal failure group. The clinical course of these patients is well illustrated by the last three cases in this series. Varying degrees of anuria or oliguria and jaundice are commonly present. Signs of pulmonary edema and congestive heart failure appear sometime after the 4th or 5th day, and the patient usually dies around the 5th to the 12th day. The kidneys show a "lower nephron nephrosis,"[29] and the liver shows foci of centrilobular necrosis.

The marked pulmonary edema, bilateral pleural effusion, and passive hyperemia of the liver, which were present in Cases 6, 7, and 8, were probably caused by congestive heart failure. Friedberg[52] and other authors§ have emphasized the dangers of administration of excess amounts of parenteral fluid to anuric patients and believe that congestive heart failure results from the hypervolemia induced by the parenteral fluid and is the commonest cause of death in this type of patient. The fluid balance charts on Cases 7 and 8 show that such a mechanism probably operated in these two patients.

Histologically, the lung changes are similar to those sometimes found in uremia[73] and are comparable to those recently described by Umiker and Pearce[64] in 27 cases of fatal $CCl_4$ poisoning. The retention of normal and abnormal metabolites in uremia seems to produce a fairly specific histologic picture in the lung. The relationship of uremia to pulmonary edema has not been established.

The anesthetic group consists of the occasional patient who dies while being exposed to a high concentration of the drug, usually by inhalation. Few clinicopathologic studies on this group have been reported. The pathologic findings in two cases observed by Moon[48] are similar to those noted in Case 1 in this series. These patients usually die before observable pathologic changes develop. The existence of an anesthetic group could have been predicted on the basis of the experimental work of von Oettingen, Powell, Sharpless, Alford, and Pecora[42] in dogs. They have shown that $CCl_4$, of all the chlorinated methanes, has the greatest depressant action on the medullary centers and that, because of this prompt depressant action, $CCl_4$ is a poorer anesthetic than chloroform ($CHCl_3$).

§ References 41, 53, and 72.

The clinical course in the fulminant group (Cases 2 to 5) is rapid, with death in 96 hours or less. Such cases are unusual, as shown by the fact that only 3 of the 75 cases of $CCl_4$ poisoning on file at the Armed Forces Institute of Pathology fall into this category. These patients all had a uniformly stormy clinical course, characterized by disorientation, often with coma; prolonged nausea and vomiting, and shock. Anuria was not a part of the clinical picture, and jaundice supervened only in the last two patients, who lived 60 hours or more. The NPN was elevated in three of the patients on whom this determination was performed, and the creatinine was also elevated on the first of these. The $CCl_4$ was inhaled in less than narcotic concentration by Patient 2, and Patients 3, 4, and 5 swallowed the drug, usually by accident. At postmortem examination only two organs were visibly involved, the liver and the kidney. The liver was the seat of fat infiltration and massive central, midzonal, and partially periportal necrosis, and the kidney contained a striking amount of fat in a basal position in the renal tubules, a lesion which Allen [74] calls fat nephrosis.

The most remarkable finding in these patients is the extensive, almost complete, destruction of the liver, and it is probable that they died in acute hepatic failure, perhaps hepatic coma (summaries of Cases 2, 3, and 5). A better designation than fulminant for this group might therefore be hepatic failure. However, the clinical histories and laboratory work-ups are so scanty that the presence of hepatic failure as a predominant finding cannot be definitely established.

It is not known why such an extensive degree of hepatic necrosis develops in these patients. A dose-response relationship is apparently not the cause, as all the patients in this series, except for Patient 1, were exposed to approximately the same dose of $CCl_4$ (probably not more than 3 to 8 cc.), with quite different responses. Also, much larger doses have been administered to many patients without serious consequences. Lamson, Minot, and Robbins [3] report that 10 to 15 cc. of $CCl_4$ has been given to thousands of patients as treatment for ancylostomiasis and that as much as 40 cc. has been given to some humans without production of serious signs and symptoms. Gardner, Grove, Gustafson, Mauri, Thompson, Wells, and Lamson [71] have given as much as 250 cc. of $CCl_4$ to a normal dog without ill effect. However, these same investigators report that administration of as little as 1 cc. of $CCl_4$ per kilogram of body weight to a dog along with 4 cc. of ethyl alcohol per kilogram of body weight is often fatal and is associated with extensive destruction of liver. Smyth, Smyth, and Carpenter [75] have made similar observations in guinea pigs.

All the patients in this series were moderate to heavy drinkers (except Patient 4, for whom this information is not recorded), as are the patients in most of the fatal cases reported in the literature,‖ and chronic alcoholism or ingestion of alcohol at the time of exposure is probably related to the development of fatal poisoning, especially in the renal failure and fulminant groups. The nature of the material used in this study makes it impossible to correlate the alcoholic intake of the patients to necrosis in the liver. However, since ethyl alcohol and $CCl_4$ are infinitely miscible and $CCl_4$ and water are essentially insoluble, it is possible that patients with high levels of blood alcohol may develop more extensive liver necrosis than patients with low levels of alcohol because of the possibility of alcohol carrying greater quantities of $CCl_4$ to the target tissues. Also, the presence of alcohol in the gastrointestinal tract may enhance absorption of $CCl_4$. At any rate, alcoholism or alcohol ingestion seems to be related in some way to fatal $CCl_4$ poisoning.

The renal lesion in the patients in the fulminant group consists essentially of large vacuoles of neutral fat in a basal position in the proximal tubular cells. Reticulum stains show the intact tubular basement membrane. The supranuclear cytoplasm contains rare fat droplets. The area occupied by the fat is

‖ References 12, 13, 15, 30, 35, 37, 38, 48, 49, 51, 54, and 59.

the portion of the proximal tubule which contains the rodlets or mitochondria. Material fixed properly to demonstrate these structures was not available, and it is possible that the observed fat may come from these lipid-containing organoids. The relation of fat nephrosis to renal function is unknown, though it should be noted that three of the four patients had an NPN of over 70 mg. per 100 cc.

Fat nephrosis occurs uniformly in phosphorus poisoning, and, according to Allen,[74] it has been noted in some cases of chloroform, tetrachloroethane, chloral hydrate, bromoform, and iodoform poisoning. It has also been observed in some cases of diabetes with hypercholesterolemia. Lucké and Mallory [76] found fat nephrosis in cases of fulminant acute infectious hepatitis and believe that it is a storage phenomenon associated with the extensive necrosis and fat infiltration of the liver which occurs in this type of hepatitis. In 39 cases which they analyzed in an attempt to correlate survival time with degree of fat nephrosis, the fat nephrosis was more marked in those patients who survived less than six days. The time relationship noted by Lucké and Mallory is also present in this series and is evidence for the close relationship between the presence of extensive fat and necrosis in the liver and fat nephrosis in the kidney.

The presence of minimal findings which could be interpreted as lower nephron nephrosis in two of the four fulminant cases may mean that, had the patients survived the hepatic injury, they would have developed renal failure. This would lead to the conclusion that fat nephrosis and lower nephron nephrosis are probably unrelated lesions.

Only a few studies have been directed at the nephrotoxic action of $CCl_4$ in the experimental animal. None of these studies, including those of Jennings and Kearns [77]; Oliver, MacDowell, and Tracy,[67] and György, Seifter, Tomarelli, and Goldblatt,[78] have revealed lesions analogous to fat nephrosis.

SUMMARY

Eight cases of fatal $CCl_4$ poisoning are reported. On the basis of clinical and pathologic findings the cases are grouped into anesthetic, renal failure, and fulminant (probably hepatic failure) groups.

Special emphasis is placed on the fulminant group, consisting of four patients who died within 96 hours of exposure to this poison, as this group has not been described previously. These cases are characterized by a rapid clinical course, fat infiltration and massive necrosis of the liver, and fat nephrosis.

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# Cholesterol Content of Aorta in Relation to Severity of Atherosclerosis

**Studies During Progression and Retrogression of Experimental Lesions**

GARDNER C. McMILLAN, M.D.
LOUIS HORLICK, M.D.
and
G. LYMAN DUFF, M.D., Montreal, Canada

While there are many studies of the progression or development of experimental cholesterol atherosclerosis in the rabbit, there are relatively few that are concerned either with its retrogression or with the relation of the cholesterol content of the experimental lesions to their apparent severity. The experiment reported here represents a systematic investigation of the content of cholesterol in relation to the severity of the aortic lesions of atherosclerosis observed in rabbits after three months of feeding with cholesterol and after periods of regression of from two weeks to six months following the cessation of cholesterol feeding.

## MATERIALS AND METHODS

The animals employed were New Zealand white pedigreed rabbits, weighing between 2 and 3 kg. at the time cholesterol feeding was begun. Cholesterol was administered with the food in a dose of 1 gm. daily for 6 days each week during a period of 90 days. The food was prepared by oiling 93 gm. of rabbit food pellets with 6 gm. of corn oil and coating them with 1 gm. of powdered cholesterol.

Seven rabbits were fed on ordinary rabbit food,* without oil or cholesterol, for two months. They were then killed, and their normal aortas were submitted to chemical analysis. Thirty-nine rabbits were fed cholesterol in the manner detailed above. They were killed after 90 days of feeding with cholesterol. A further 62 rabbits were fed cholesterol in the same manner. On completing 90 days of cholesterol feeding they were placed on a standard cholesterol-free diet of pellets for from two weeks to six months, during which time it was presumed that the aortic lesions developed during the cholesterol feeding period would retrogress.

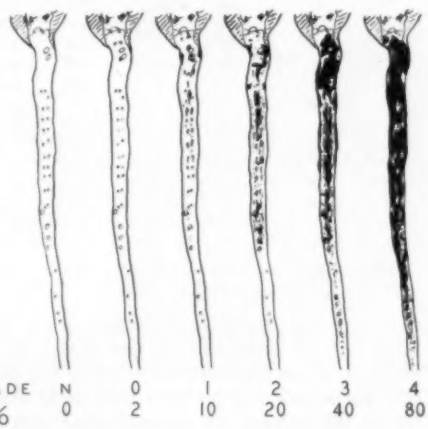


Diagram of a normal aorta, *N*, with no intimal lesions and of five aortas showing Grades 0, 1, 2, 3, and 4 atherosclerosis, with a visually estimated involvement of approximately 2%, 10%, 20%, 40%, and 80% of the intimal surface area by atherosclerotic lesions. The atherosclerotic lesions are illustrated in black. The aortic conus and adjacent myocardium, the aortic valve, and the ventricular aspect of the mitral valve are drawn at the top of the diagram.

Groups of four to six rabbits were killed every two weeks. The details of the feeding and killing schedule were so arranged that at every biweekly killing period several rabbits that had just completed 90 days of cholesterol feeding, four or more rabbits that had been in the retrogressive phase for

From the Department of Pathology, Pathological Institute, McGill University.

Assisted by a grant from the Canadian Life Insurance Officers Association.

James Douglas Research Fellow in Pathology (Dr. Horlick).

* "Miracle" Rabbit Pellets, The Ogilvie Flour Mills Co., Ltd., Montreal, Canada.

$X$ weeks, and four or more rabbits that had been in the retrogressive phase for $X$ weeks plus three months were killed. In this manner lesions of different duration were examined on the same day. In an additional experiment 17 rabbits were fed cholesterol in the same manner as the previous group, except that the dose was 0.5 gm. during the first six weeks and 1.0 gm. during the subsequent six weeks. After an interval of 19 weeks of retrogression, during which time no cholesterol was given to the rabbits, they were killed.

On the completion of the experimental periods the rabbits were killed and examined at autopsy. The aorta was examined visually, and the severity of atherosclerosis was graded. The cholesterol content of the aorta was then determined chemically.

In practice, although the severity of any one grade (except Grade 0) is half that of the following one, it is not always a simple matter to classify properly a given specimen, because of the variable distribution of the lesions and because of intermediate degrees of involvement. Furthermore, while the scheme does not contain any provision for consideration of the depth or thickness of the atherosclerotic lesions, this element nevertheless intrudes and influences the subjective grading of the lesions.

After grading, the fresh aorta was freed from the heart and carefully cleaned of its adventitia by

TABLE 1.—*Grade of Atherosclerosis and Cholesterol Content of Aorta After Ninety Days of Cholesterol Feeding*

| Cholesterol Content of Aorta, Mg./Aorta | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal Controls, 0% | | Grade Zero, Less Than 2% | | Grade 1, 10% | | Grade 2, 20% | | Grade 3, 40% | | Grade 4, 80% | |
| Free | Total | Free | Total | Free | Total | Free | Total | Free | Total | Free | Total |
| — | 0.90 | 1.00 | 1.60 | 1.50 | 2.75 | 3.10 | 8.20 | 2.60 | 4.15 | 4.25 | 16.76 |
| — | 0.95 | 1.00 | 2.00 | 1.85 | 3.25 | 3.50 | 8.75 | 2.52 | 4.60 | 9.87 | 22.25 |
| — | 0.95 | 1.10 | 2.25 | 1.86 | 5.00 | 3.85 | 13.50 | 2.88 | 8.10 | 7.60 | 24.26 |
| — | 1.10 | 1.37 | 2.25 | 3.25 | 5.87 | 6.48 | 18.34 | 4.60 | 11.00 | 6.83 | 26.59 |
| — | 1.20 | 1.37 | 3.00 | 3.37 | 6.62 | .... | .... | 4.00 | 11.26 | 9.76 | 28.15 |
| — | 1.30 | .... | .... | 4.25 | 9.75 | .... | .... | 11.47 | 13.81 | 8.67 | 28.53 |
| — | 1.40 | .... | .... | .... | .... | .... | .... | 5.15 | 15.10 | 9.84 | 28.53 |
| .. | .... | .... | .... | .... | .... | .... | .... | 6.26 | 18.00 | — | 31.13 |
| .. | .... | .... | .... | .... | .... | .... | .... | 6.48 | 20.62 | 14.69 | 40.47 |
| .. | .... | .... | .... | .... | .... | .... | .... | 8.75 | 20.85 | 14.17 | 50.71 |
| .. | .... | .... | .... | .... | .... | .... | .... | 8.75 | 24.25 | .... | ..... |
| .. | .... | .... | .... | .... | .... | .... | .... | 8.63 | 28.93 | .... | ..... |
| .. | .... | .... | .... | .... | .... | .... | .... | 11.25 | 30.20 | .... | ..... |
| .. | .... | .... | .... | .... | .... | .... | .... | 13.66 | 34.17 | .... | ..... |
| Mean | 1.13 | 1.17 | 2.22 | 2.68 | 5.54 | 4.23 | 12.20 | 6.89 | 17.50 | 9.54 | 29.68 |

The method of grading the aortas for the degree of severity of atherosclerosis was similar, but not identical, to one that has been in use in this laboratory for several years.[1] The entire aorta was removed and opened. Its intimal surface was scrutinized, and the severity of atherosclerosis was graded on a scale of 0 to 4. On this scale 0 represents either no microscopically visible lesions or only very minute ones and is arbitrarily considered to represent an involvement of less than 2% of the total surface of the intima with atherosclerotic lesions. Grade 1 represents an average involvement of about 10%, Grade 2 of about 20%, Grade 3 of about 40%, and Grade 4 of about 80% of the intimal surface of the aorta by atherosclerotic lesions (Fig.).

The percentages detailed above and illustrated in the Figure are meant to express ideal reference values rather than real ones.

means of fine forceps. Occasionally a plane of cleavage in the media could be found and the adventitia stripped away. The fresh aorta was then placed in a small jar containing absolute ethyl alcohol, to await subsequent extraction.

The aorta was chopped into pieces about 1 cm. long and a millimeter or two in width and extracted with 100 cc. of hot absolute ethyl alcohol (including the alcohol in which it had been originally stored) for six hours in a continuous extraction apparatus. It was then exposed to 25 cc. of ethyl ether at room temperature overnight. The two extracts were combined, brought to volume by evaporation, and stored in a stoppered flask.

A 2 or 4 ml. aliquot of the crude extract was evaporated to a volume of about 0.5 ml. and restored to an approximate volume of 2 ml. with a mixture of equal parts of acetone and alcohol. The free and total cholesterol content of the sample was

TABLE 2.—*Grade of Atherosclerosis and Cholesterol Content of Aorta During Retrogression*

| Weeks of Retrogression | Cholesterol Content of Aorta, Mg./Aorta | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Grade 0, Less Than 2% | | Grade 1, 10% | | Grade 2, 20% | | Grade 3, 40% | | Grade 4, 80% | |
| | Free | Total | Free | Total | Free | Total | Free | Total | Free | Total |
| 2 and 4 | .... | .... | 1.70 | 2.75 | .... | ..... | 18.00 | 40.00 | 5.35 | 11.75 |
| | .... | .... | 1.75 | 3.50 | .... | ..... | 13.56 | 41.50 | 26.50 | 55.00 |
| | .... | .... | 1.88 | 4.00 | .... | ..... | ..... | ..... | ..... | ..... |
| | .... | .... | 2.00 | 4.28 | .... | ..... | ..... | ..... | ..... | ..... |
| | .... | .... | 4.26 | 8.50 | .... | ..... | ..... | ..... | ..... | ..... |
| 6 and 8 | 0.63 | 1.38 | 1.50 | 2.75 | 2.00 | 4.35 | ..... | ..... | 6.12 | 15.87 |
| | 0.76 | 1.50 | 1.75 | 3.87 | 5.62 | 14.62 | ..... | ..... | ..... | ..... |
| | 0.87 | 1.62 | 2.00 | 6.75 | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.62 | 3.00 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| 10 and 12 | 0.60 | 1.00 | 1.35 | 4.42 | 3.50 | 8.00 | ..... | ..... | 22.85 | 40.00 |
| | 0.35 | 1.25 | 3.25 | 6.25 | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.00 | 1.60 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.10 | 1.62 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.00 | 1.63 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.25 | 1.63 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| | 1.25 | 1.75 | .... | .... | .... | ..... | ..... | ..... | ..... | ..... |
| 14 and 16 | .... | .... | 5.60 | 12.00 | 3.75 | 10.10 | 6.00 | 11.70 | 18.25 | 31.00 |
| | .... | .... | .... | .... | 4.50 | 10.74 | 5.00 | 14.74 | 33.85 | 54.10 |
| | .... | .... | .... | .... | 8.10 | 13.50 | 9.35 | 16.74 | 44.75 | 83.00 |
| | .... | .... | .... | .... | .... | ..... | 10.10 | 24.25 | ..... | ..... |
| 18 | .... | .... | .... | .... | 5.50 | 11.76 | 7.36 | 14.00 | ..... | ..... |
| | .... | .... | .... | .... | 6.52 | 12.00 | ..... | ..... | ..... | ..... |
| | .... | .... | 3.50 | 10.00 | .... | ..... | ..... | ..... | ..... | ..... |
| 19 | .... | .... | .... | .... | 3.40 | 7.10 | 11.00 | 18.30 | 13.90 | 20.75 |
| | .... | .... | .... | .... | 5.20 | 8.70 | 12.20 | 23.10 | 14.50 | 21.00 |
| | .... | .... | .... | .... | 6.10 | 9.90 | 17.00 | 25.30 | 16.90 | 28.30 |
| | .... | .... | .... | .... | 3.50 | 10.30 | 29.60 | 42.00 | 29.60 | 52.90 |
| | .... | .... | .... | .... | 7.00 | 11.60 | ..... | ..... | 44.10 | 57.90 |
| | .... | .... | .... | .... | 7.10 | 11.60 | ..... | ..... | ..... | ..... |
| | .... | .... | .... | .... | 10.20 | 15.40 | ..... | ..... | ..... | ..... |
| | .... | .... | .... | .... | 14.70 | 24.50 | ..... | ..... | ..... | ..... |
| 20 and 22 | 0.87 | 1.50 | 2.25 | 4.75 | 15.12 | 23.25 | ..... | ..... | 11.75 | 22.87 |
| | .... | .... | 4.25 | 8.25 | 16.37 | 25.00 | ..... | ..... | 24.25 | 39.50 |
| | .... | .... | .... | .... | .... | ..... | ..... | ..... | 29.00 | 48.50 |
| 24 and 26 | 1.00 | 1.75 | 5.12 | 10.50 | 1.35 | 3.25 | ..... | ..... | 21.85 | 33.25 |
| | .... | .... | .... | .... | 2.60 | 6.60 | ..... | ..... | 27.37 | 36.50 |
| | .... | .... | .... | .... | 7.00 | 12.25 | ..... | ..... | 26.50 | 43.25 |
| | .... | .... | .... | .... | .... | ..... | ..... | ..... | 53.70 | 81.50 |
| Mean | 0.95 | 1.63 | 2.81 | 6.17 | 6.62 | 12.12 | 12.62 | 24.69 | 24.79 | 40.89 |

then estimated, by the Schoenheimer-Sperry method, and was expressed as the absolute number of milligrams of cholesterol contained in the aorta.

## OBSERVATIONS

The data derived from the study of the 7 normal rabbits that were not fed cholesterol and the 39 rabbits fed cholesterol for 90 days are presented in Table 1.

The data relating to the free and total cholesterol content of the aorta and the grading of the aortic atherosclerosis observed in the retrogression experiment are summarized in Tables 2 and 3. The data relating to the 17 animals that were allowed a period of 19 weeks of retrogression have been included in Tables 2 and 3; it is to be noted that they were not fed cholesterol in exactly the same amount as the other 62 rabbits. It should be remarked that a progressively decreasing de-

TABLE 3.—*Average Content of Cholesterol in Aorta During Retrogression*

| Weeks of Retrogression | Rabbits, No. | Mean Cholesterol Content, Mg./Aorta | | |
|---|---|---|---|---|
| | | Free | Total | Ester (% of Total) |
| 0 (Table 1) | 38 | 5.82 | 15.91 | 62.8 |
| 2 and 4 | 9 | 8.30 | 19.03 | 56.4 |
| 6 and 8 | 10 | 2.29 | 5.57 | 58.9 |
| 10 and 12 | 11 | 3.41 | 6.29 | 45.8 |
| 14 and 16 | 11 | 13.57 | 25.62 | 47.0 |
| 18 | 4 | 5.72 | 11.94 | 52.1 |
| 19 | 17 | 14.47 | 22.86 | 36.7 |
| 20 and 22 | 8 | 12.98 | 21.70 | 40.1 |
| 24 and 26 | 9 | 16.28 | 25.43 | 36.0 |

gree of hypercholesteremia persists for some time after the completion of cholesterol feeding and the withdrawal of cholesterinized food. Normal serum cholesterol values are regained only eight or more weeks after the rabbits have been returned to a normal diet. A progression of the lesions beyond those observed after 90 days of cholesterol feeding (0 weeks of retrogression; Table 1) is to be expected, therefore.

## COMMENT

The experimental data derived from the study of those rabbits that were fed cholesterol for three months and then returned to a cholesterol-free diet for periods of from two to six months provide two conclusions of interest. The data demonstrate that the quantity of cholesterol in the aorta does not decrease during a period of potential retrogression of as long as a half year. This result is somewhat unexpected, for it was stated by Anitschkow,† many years ago, that a definite retrogression of lesions with a disappearance of much lipid occurred in the aortic atherosclerotic plaques of rabbits when they were allowed to retrogress for about one and one-half to three years. The data reported here fail to detect the commencement of this process. On the other hand, a morphological reorganization of the lesions [2] occurs rapidly and can be detected both grossly and microscopically. In our experience, lesions allowed to retrogress for two or three months or longer show a considerable amount of fibroblastic proliferation beneath the endothelium as well as necrosis in their centers, with the formation of large pools of extracellular lipid material. The morphological changes in the structure of the atherosclerotic lesions incorporate the lipid of the lesions deeply beneath fibrous tissue and convey the erroneous impression, on both macroscopic and microscopic examination, that the lipid content of the lesions has undergone a regression of major extent. It is apparent that this morphological reorganization in the present experiment was not accompanied by a decrease in the total chemical content of cholesterol in the aorta. On the contrary, the aortas contained more cholesterol, on the average, after retrogression than they contained immediately on the completion of 90 days of cholesterol feeding. The latter observation is explained, in part at least, by the fact that the rabbits remained in a state of marked hypercholesterolemia for several weeks following the cessation of cholesterol feeding. It is obvious that the lesions have not regressed, either in terms of visual grading or in the sense of decreasing their total content of cholesterol. Anitschkow [2] remarked that the anatomical retrogression of the lesions was an exceedingly slow process that was not apparent after relatively short periods of regression, such as those employed in the present experiment. He also noted that, while the lipid content of the larger lesions did not disappear entirely, that of the smaller lesions, especially in the proximal aorta, might regress completely. It is probable that a more prolonged experiment, with an analysis of the lipid content of the thoracic aorta in particular, would illustrate the chemical phenomena associated with the regression of experimental cholesterol atherosclerosis in the rabbit. Since an appreciable regression of well-developed lesions does not occur spontaneously during a period of as long as six months following the cessation of cholesterol feeding, any well-founded experiment that demonstrates the experimental induction of retrogression during this period acquires immediate significance.

It is of interest to note that there was a tendency for the relative proportions of free and ester cholesterol in the aorta to change during the course of the experiment. The groups killed after 90 days of feeding and no period of regression (Table 1) and after from two to eight weeks of retrogression (Table 3) possess esterified cholesterol values that are 50 to 60% of the total aortic cholesterol. Those killed from the 10th to 18th weeks yield values in which the ester cholesterol is 40 to 50% of the total, while those killed after 19 to 26 weeks of retro-

† References 2 and 3.

gression have a content of esterified cholesterol that is 35 to 40% of the total cholesterol present in the aorta. This progressive decrease in ester cholesterol, with a corresponding increase in free cholesterol content, is not sufficiently constant to be regarded as anything more than a trend in the present experiment; however, it may be noted that it is associated with increasing time periods of retrogression rather than with the total content of cholesterol contained in the aortas. It was also observed that the mean aortic cholesterol values associated with the various grades of lesions tended to be somewhat higher in those animals killed during retrogression than in those rabbits killed immediately on the cessation of cholesterol feeding. This was evident in the case of lesions of Grade 3 and Grade 4 severity.

Tables 1 and 2 demonstrate the great variation that occurs in the amount of atherosclerosis that develops in individual rabbits fed cholesterol. An appreciable number of rabbits was found to have no atherosclerotic lesions, or only minimal ones. This variability was apparent not only in those animals killed on or soon after the cessation of cholesterol feeding but also in those killed during later periods of the experiment. The experiment has focused attention sharply on methods of assessing the severity of atherosclerosis. While there is a close linear correlation between the grades of severity of atherosclerosis and their respective mean aortic contents of cholesterol (Table 1), there is also a very wide spread of individual aortic cholesterol contents within any one grade, together with a considerable overlap between adjacent grades. Indeed, this overlap is so great that the lowest content of a Grade 3 lesion was about equal to that of an average Grade 1 lesion, and the lowest content of a Grade 4 lesion was less than that of the greatest Grade 2 lesion and about equal to that of an average Grade 3 lesion. The visual method of estimating the amount of the intima that is involved by lesions is obviously subject to various errors. Some of these are personal to the individual observers; others depend on the association of the experimental lesions with those of spontaneous medial arteriosclerosis of the aortic wall; some are related to the variability in the thickness or elevation of the intimal lesions and to the amount of intimal fibrosis, and others are related to the considerable variation in the total surface area of the aorta in different rabbits. Furthermore, the procedure of grouping aortas into arbitrary crude classes or grades has nothing to recommend it other than custom and convenience. It is considered that visual grading of the severity of experimental atherosclerosis is a justifiable method only if the numbers of animals examined are large enough to avoid sampling errors and to permit adequate statistical treatment, or if the difference in the severity of atherosclerosis between experimental and control animals is large. If, as is often the case, only a minor difference exists and the number of animals is small, grading is not a suitable procedure, since it may lead to both "false positive" and "false negative" conclusions.

The chemical content of cholesterol in the aorta is considered to be a useful index of the severity of atherosclerosis. It is objective rather than subjective, and it is a more sensitive measure of minor differences. Nevertheless, it is by no means considered to be an ideal method of assessing atherosclerosis. Chemical determinations of the severity of atherosclerosis suffer from the fact that it is technically impossible to dissect an aorta accurately and express its cholesterol content per unit weight of aorta or aortic tissue. The content per aorta is probably a more meaningful figure than the content per gram of wet or dry weight of dissected tissue. However, aortas vary considerably in size. It follows, therefore, that the cholesterol content of two aortas of different size cannot be compared with accuracy if expressed as content per aorta. The more logical scheme would be to express the cholesterol content in terms of milligrams of cholesterol per unit of surface area of the intima of the aorta, but we

made no measurements of surface area in this experiment.

The chemical data recorded in the present experiment do not necessarily constitute a more valid expression of the severity of the lesions of aortic atherosclerosis than the visual impression of their severity. Inasmuch as each method of assessment, as currently practiced, is subject to large inherent errors, it is not expected that there will be a close correlation between them in all cases. Indeed, it is apparent that an accurate assessment of the lesions will only be obtained by characterizing them both chemically and morphologically as completely as possible. We regard it as important that the inadequacies of both methods should be widely recognized among experimentalists.

## SUMMARY

Experiments were done in which rabbits were fed cholesterol until they developed aortic atherosclerosis. Some of the rabbits were killed immediately on the cessation of cholesterol feeding, and others were returned to a normal diet, free from cholesterol, for as long as six months. Their aortas were submitted to visual grading of the severity of cholesterol atherosclerosis and to chemical analysis of their cholesterol content. It was found that cholesterol was not lost from the atherosclerotic aorta during a period of retrogression of as long as six months, even though the lesions underwent a morphological reorganization that might be construed as a regression. It was also observed that there was a relative increase in free cholesterol or decrease in esterified cholesterol in the lesions during the period following the cessation of cholesterol feeding.

The data obtained from the visual grading of the severity of aortic atherosclerotic lesions are compared with those obtained by chemical analysis of the cholesterol content of the aorta. There is only a relatively crude and variable correlation. The difficulties of the assessment of the severity of experimental atherosclerosis are discussed briefly. It is concluded that both the visual and chemical methods of assessment of the severity of experimental atherosclerosis are subject to large inherent errors. The inadequacies of both methods, together with the great natural variation that occurs in the amount of atherosclerosis which develops in individual rabbits fed cholesterol, compel the investigator to use adequate numbers of rabbits and a suitable contemporaneous control series to reduce sampling, procedural, and observational errors and to minimize the possibility of false interpretation.

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# Critical Analysis of Two Hundred Twenty-One Thyroid Glands

**Study of Thyroid Glands Obtained at Necropsy in Colorado**

OSMAN H. HULL, M.D., San Francisco

The thyroid gland has been studied and reported upon in many parts of the United States and in other countries. No investigation of the type to be described in detail has been made for the Rocky Mountain area. Since this is a mountainous, fresh-water area and a potential endemic goiter region, thyroid lesions should be common enough to yield abundant material for thorough and critical analysis. Necropsy material affords entire thyroid glands for study and more nearly represents the incidence of possible lesions in the population, especially in persons over 20 years of age. Colorado is representative of the Rocky Mountain states, with a central location and the largest population.

## REVIEW OF THE LITERATURE

The weight of the thyroid gland has been studied by Jaffe [1] in Chicago, Rice [2] in Minnesota, Kolnitz and Remington [3] in South Carolina, Hellwig [4] in Kansas, Nolan [5] in Minneapolis, and Hazard and Kaufman [6] in Cleveland. Rice,[2] Hellwig,[4] and Kolnitz and Remington [3] have adequately reviewed similar studies in Europe and have compared their findings to those observed primarily in Germany and Switzerland. The incidence of nodules in the thyroid gland has been reported by some of these men and has also been reported by Schlesinger, Gargill, and Saxe [7] and by Coffey, Amoroso, and Mazzara.[8] Carcinoma in thyroid glands examined at necropsy has been discussed by those studying nodules of all types and, specifically, by Vander Laan [9] and by Beal, Scholnick, and Stevens.[10] Carcinoma has usually been discussed in relation to surgical specimens by others; where pertinent, such material will be included in this study for purposes of comparison. Graham [11]; Hazard, Crile, and Dempsey [12]; Frazell and Foote [13]; Cole, Majarakis, and Slaughter [14]; Cope, Dobyns, Hamlin, and Hopkirk [15]; Crotti [16]; Beahrs, Pemberton, and Black,[17] and many others have recorded valuable data concerning the occurrence of carcinoma in the thyroid gland. Other thyroid gland lesions to be recorded and discussed with relation to previous studies are the incidence of squamous metaplasia, of the Delphian node, of cancer metastatic to the thyroid gland, of the absence of the thyroid gland isthmus, of the presence of a pyramidal lobe, and of the presence of parathyroid glands buried within thyroid gland tissue. Also discussed is the correlation of antemortem physical findings and thyroid gland lesions found at necropsy. Problems not to be discussed because of lack of adequate material in this series are diseases of the thyroid gland in children in the first decade of life, hyperthyroidism, and thyroiditis.

## METHODS AND MATERIALS

During 1952 and 1953 a total of 221 thyroid glands was obtained at necropsy at the Colorado General Hospital. The patients admitted to this hospital are from all counties of the state. In 175, or 79.2%, of the cases studied the patient had lived in Colorado for 11 years or more. In 17 cases the patient had

Present address: St. Francis Hospital, 900 Hyde St.

From the Department of Pathology, University of Colorado School of Medicine, Denver, James B. McNaught, M.D., Head of the Department of Pathology.

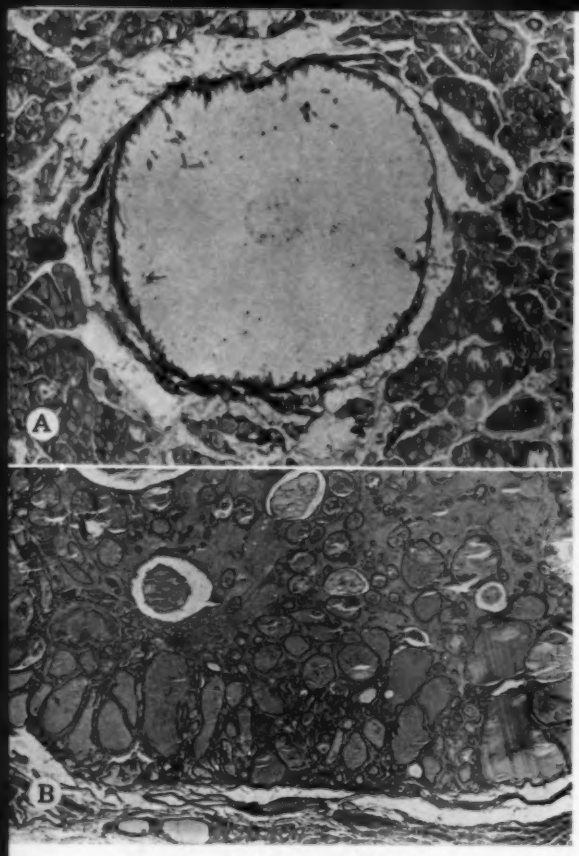
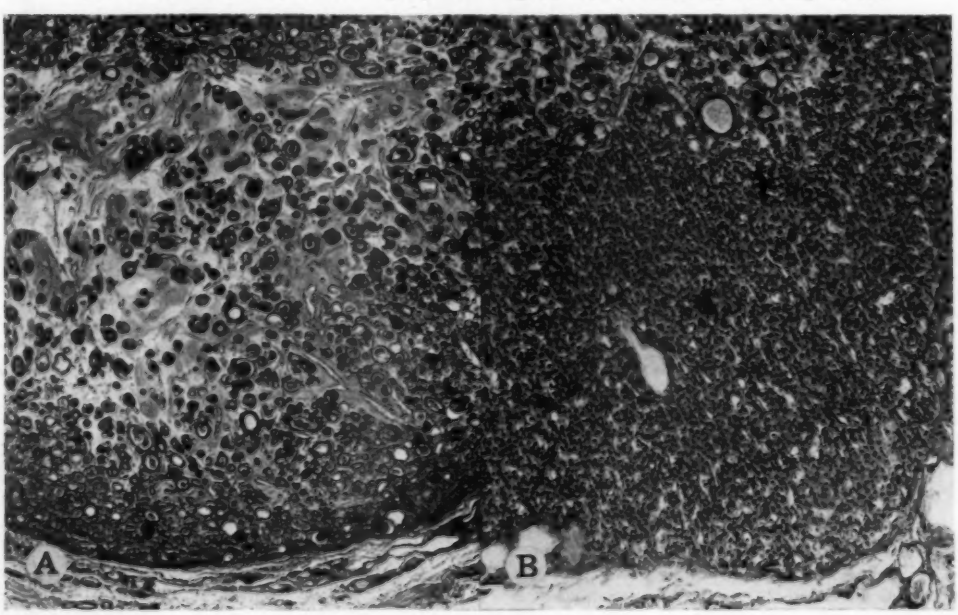



Fig. 1.—*A*, follicular nodule formed by a single giant follicle, probably due to simple hypertrophy; reduced ¼ from mag. × 15. *B*, follicular nodule composed of multiple adult follicles; reduced ⅙ from mag. × 40.

lived in Colorado for 6 to 10 years, in 19 cases 1 to 5 years, and in 10 cases for less than one year. Neck organs were removed in a single mass immediately after arterial embalming of the head and neck. The thyroid gland was dissected free and weighed. The relationship of parathyroid glands, lymph nodes, pyramidal lobe, and accessory thyroid tissue was determined for each specimen at that time. A few children in the first decade of life and a few adults who had had thyroidectomies were eliminated from consideration. Cases were taken consecutively, insofar as possible, although this was not always feasible owing to technical difficulties and the limitations of autopsy permission in several cases. After fixation in Zenker's fluid, washing in water, and immersion in 70% ethyl alcohol, the thyroid glands were sketched and cut in 2 mm. step sections. The location and size of all gross lesions were recorded on the sketch. Microscopic sections were made of all lesions found. Two sections were made of each lobe and of the isthmus at random in those glands with no gross abnormalities. In addition, sections were made of the connective and fat tissues immediately anterior and superior to the isthmus, of any apparent accessory thyroid tissue usually located adjacent to the inferior portions of the lobes and of the isthmus, and of several cervical lymph nodes adjacent to the thyroid gland. The tissues were treated with the alcohol-dioxane techniques, embedded in paraffin, cut at 8μ, and stained with hematoxylin and eosin. After microscopic examination of the sections, the type of nodules found

Fig. 2.—Fetal nodule. *A*, reduced slightly from mag. × 40; *B*, reduced ⅙ from mag. × 100.



and other characteristics were recorded on the sketch. The charts of the patients were searched for evidence of clinical thyroid disease. Results of physical examination of the thyroid gland were abstracted. The chief cause of death was tabulated for each case.

### DEFINITION OF TERMS

*Follicular Nodule.*—This nodule is the type referred to by many as "colloid nodule." In this type of nodule the dominant unit of structure was a large, thin-walled follicle, filled with colloid and lined by flattened cuboidal epithelium. Nuclei were small, round, central, and uniform and had fine, slightly peripherally arranged chromatin. Some of these nodules consisted of a single giant follicle, probably due to simple hypertrophy (Fig. 1*A*). Most of these nodules were composed of multiple follicles, varying considerably in size and shape, always filled with colloid, and sometimes showing remaining spurs, presumably from previous hyperplasia (Fig. 1*B*). These nodules were frequently accompanied by fibrosis, calcification, and evidence of old hemorrhage. A blending of adult and fetal follicles was often seen. Nodules consisting predominantly of enlarged adult follicles were classified as follicular nodules.

*Fetal Nodule.*—In nodules of this type the dominant unit of structure was a small follicle with little or no colloid. The follicles at the periphery of the nodules were closely packed. The follicles at the center of the nodules often were loosely scattered in hyalin deposited in the stroma. Lining cells of the follicles were low columnar to cuboidal. Nuclei were basal, round, uniform, with fairly heavy meshed chromatin (Fig. 2*A*, *B*). Nodules consisting mainly of fetal-like follicles were classified as fetal nodules, although adult follicles and solid cords of thyroid epithelial cells were admixed.

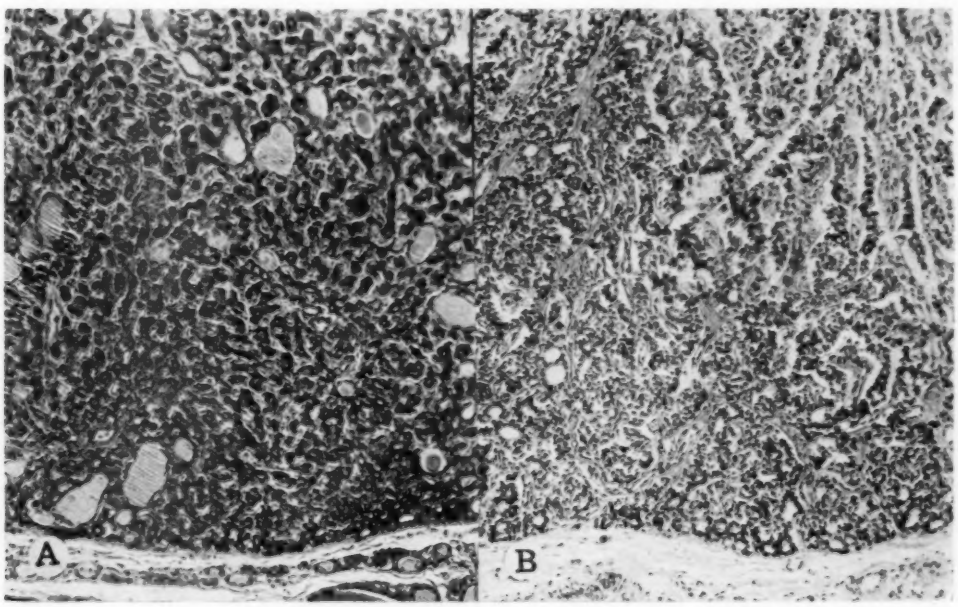



Fig. 3.—Embryonal nodule. *A*, reduced ⅙ from mag. × 90; *B*, reduced slightly from mag. × 100.

*Embryonal Nodule.*—This type of nodule was characterized by closely packed groups or cords of cells with little or no tendency to follicle formation. Cells were polyhedral to columnar, with central oval or round nuclei. Nuclear chromatin was fine and evenly distributed (Fig. 3*A*, *B*).

*Oxyphil Cell Nodule.*—This type of nodule was composed of large polyhedral cells with

abundant acidophilic cytoplasm. These cells were arranged in solid sheets and less frequently formed small follicles. Nuclei were round to oval, with a moderate amount of fine chromatin and distinct nucleoli (Fig. 4 *A, B*). This type of cell could be found in many of the previously described types of nodules. With careful search a few cells of this type could be found in most thyroid glands, especially in follicles adjacent to nodules. The cell type corresponds to that usually termed "Hürthle cell."

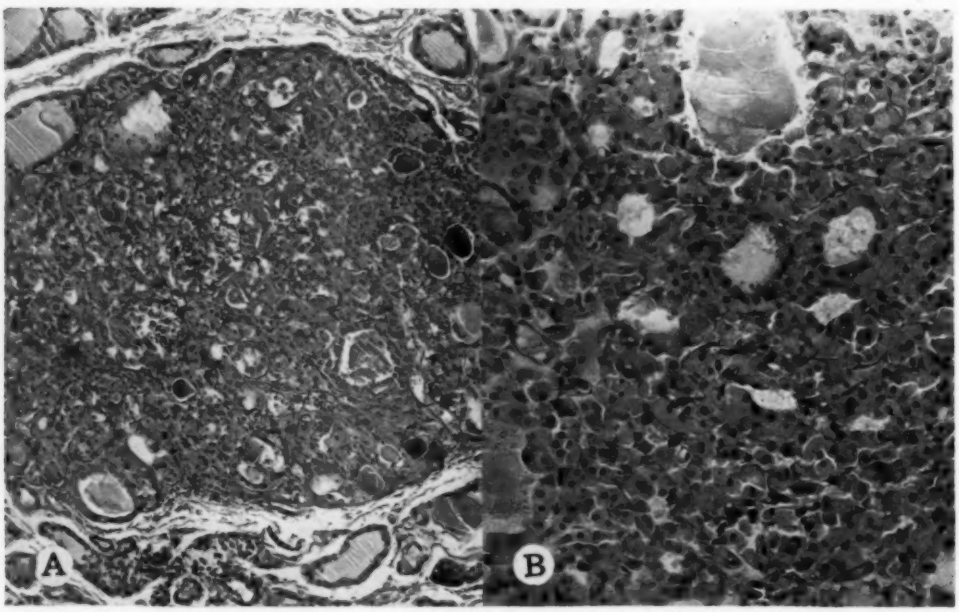



Fig. 4.—Oxyphil cell nodule. *A*, reduced slightly from mag. × 100; *B*, reduced ⅙ from mag. × 175.

## RESULTS AND COMMENT

*Weight of the Thyroid Gland.*—An analysis of the weights of all thyroid glands examined is presented in Table 1. The Table presents the information by decades and sex, as well as the extremes of thyroid weight encountered. In males, 127 thyroid glands had an average weight of 32.4 gm.; in females, 94 glands averaged 24.7 gm.

In Table 2 are analyzed the weights of thyroid glands which contained no nodules or other significant lesions and may be regarded as normal. Among 127 males, 48, or 37.8%, of the thyroid glands had no nodules and weighed an average of 19.3 gm.; among 94 females, 31, or 33.0% of the glands had no nodules and averaged 17.7 gm. in weight. A 40.5 gm. gland from one male was included in the group without nodules, but all other glands without nodules weighed 35 gm. or less. The figure of 35 gm. as the upper limit of normal adult thyroid gland weight was suggested by Arndt * and was used in the studies of Hellwig [4] and of Hazard and Kaufman.[6]

Comparison of weights found in these and other studies is made in Table 3. This analysis indicates that the average weight of normal adult thyroid glands is lower in the Rocky Mountain region than in the Great Lakes and Mississippi Valley areas, but not so low as in the coastal region of eastern South Carolina. The range of weights of 6.5 to 40.5 gm. is similar to the 7.5 to 48.5 gm. range found in South Carolina by Kolnitz and Remington,[3] but quite different from

* Arndt,[18] p. 282, cited by Hellwig.[4]

TABLE 1.—*Thyroid Gland Weight by Decades, Based on All 221 Cases*

| Decade | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Cases, No. | Av. Wt., Gm. | Extremes of Wt., Gm. | Cases, No. | Av. Wt., Gm. | Extremes of Wt., Gm. |
| 2 | 5 | 15.3 | 9-23 | 4 | 13.6 | 8-16 |
| 3 | 7 | 26.4 | 9.5-49 | 8 | 22.0 | 14.5-36.5 |
| 4 | 3 | 23.8 | 17-35 | 10 | 18.5 | 6.5-25.5 |
| 5 | 16 | 45.4 | 10-107 | 9 | 27.5 | 9.5-91 |
| 6 | 9 | 27.5 | 13-46.5 | 12 | 16.9 | 8-32 |
| 7 | 28 | 32.0 | 11-74 | 17 | 24.3 | 13-61 |
| 8 | 40 | 31.8 | 10-67 | 26 | 33.7 | 12-120 |
| 9 | 17 | 33.2 | 7.5-53 | 6 | 28.7 | 11-52 |
| 10 | 2 | 56.0 | 22-90 | 1 | 14.5 | ..... |
| 11 | 0 | .... | ..... | 1 | 47.0 | ..... |
| Totals | 127 | 32.4 | 7.5-107 | 94 | 24.7 | 6.5-120 |

the 15 to 140 gm. range found in Chicago by Jaffe.[1] The thyroid glands were weighed immediately after removal in my series and then fixed in Zenker's solution. Hazard and Kaufman [6] weighed their glands directly after removal and fixed the tissue in 10% formalin in 80% alcohol. Nolan [5] fixed his specimens in formalin, and Kolnitz and Remington [3] noted that the thyroid glands in their series were weighed unfixed after removal. The other authors cited in Table 3 did not give the methods used in their studies.

Table 4 shows the average weights of both normal and nodular thyroid glands weighing 35 gm. or less. Reflected is the increased weight added by the inclusion of the nodular glands. Thus, 49 thyroid glands in males and 47 in females contained nodules, but did not attain goitrous enlargement. Two of the three cases of incidental thyroid carcinoma found in this series were in glands weighing less than 35 gm.

TABLE 3.—*Comparison of Average Weight of Thyroid Glands Without Nodules at Autopsy*

| Author | Cases, No. | Av. Wt., Gm. |
|---|---|---|
| Jaffe,[1] Illinois, 1930 | 1,000 | 27.8 |
| Rice,[2] Minnesota, 1932 | 555 | 25.99 |
| Kolnitz and Remington,[3] South Carolina, 1933 | 150 | 15.7 |
| Hellwig,[4] Kansas, 1935 | 100 | 23.2 |
| Nolan,[5] Minnesota, 1938 | 725 | 24.4 |
| Hazard and Kaufman,[6] Ohio, 1952 | 429 | 25.9 |
| Hull, Colorado, 1954 | 221 | 18.5 |

Thyroid glands weighing more than 35 gm., and, therefore, enlarged by this arbitrary standard, are listed in Table 5. In 31, or 24.4%, of 127 males and in 16, or 17.0%, of 94 females the thyroid glands weighed more than 35 gm. This is an incidence of 21.3% for the entire study. Hellwig,[4] in

TABLE 2.—*Weight of Thyroid Glands Without Nodules, Based on Seventy-Nine Cases**

| Decade | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Cases | | Av. Wt., Gm. | Extremes of Wt., Gm. | Cases | | Av. Wt., Gm. | Extremes of Wt., Gm. |
| | No. | %† | | | No. | %† | | |
| 2 | 4 | 80.0 | 10.6 | 9-23 | 4 | 100.0 | 12.4 | 8-16 |
| 3 | 3 | 42.9 | 23.2 | 17-30.5 | 5 | 62.5 | 22.0 | 17-28.5 |
| 4 | 2 | 66.7 | 19.3 | 17-21.5 | 7 | 70.0 | 16.2 | 6.5-25.5 |
| 5 | 8 | 50.0 | 22.5 | 12-32 | 2 | 22.2 | 20.0 | 14-26 |
| 6 | 6 | 66.7 | 24.2 | 13-40.5 | 6 | 50.0 | 15.9 | 9-23 |
| 7 | 9 | 32.2 | 20.1 | 11-31.5 | 3 | 17.6 | 18.3 | 13-24 |
| 8 | 11 | 27.5 | 19.4 | 12-30 | 3 | 11.6 | 24.5 | 17.5-33 |
| 9 | 5 | 29.4 | 15.0 | 7.5-27 | 1 | 16.7 | 12.0 | ..... |
| 10 | 0 | .... | .... | ..... | 0 | .... | .... | ..... |
| 11 | 0 | .... | .... | ..... | 0 | .... | .... | ..... |
| Totals | 48 | 37.8 | 19.3 | 7.5-40.5 | 31 | 33.0 | 17.7 | 6.5-33 |

* 35.7% of total.
† The percentage following the number of cases is the percentage of the total number of cases in that decade.

TABLE 4.—*Thyroid Glands Weighing Thirty-Five Grams or Less, Based on 174 Cases*

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Cases | | Av. Wt., | Extremes of Wt., | Cases | | Av. Wt., | Extremes of Wt., |
| Decade | No. | %* | Gm. | Gm. | No. | %* | Gm. | Gm. |
| 2 | 5 | 100.0 | 15.3 | 9-23 | 4 | 100.0 | 13.6 | 8-16 |
| 3 | 5 | 71.4 | 18.9 | 9.5-30.5 | 7 | 87.5 | 21.1 | 14.5-32 |
| 4 | 3 | 100.0 | 23.8 | 17-35 | 10 | 100.0 | 18.6 | 6.5-25.5 |
| 5 | 15 | 93.8 | 24.1 | 10-34 | 8 | 88.9 | 21.4 | 9.5-27.5 |
| 6 | 7 | 77.8 | 23.8 | 13-35 | 12 | 100.0 | 16.9 | 8-23 |
| 7 | 20 | 71.4 | 19.9 | 11-35 | 13 | 76.5 | 17.3 | 13-29 |
| 8 | 27 | 67.5 | 19.5 | 10-32 | 19 | 73.1 | 23.9 | 12-33 |
| 9 | 13 | 76.5 | 19.5 | 7.5-30 | 4 | 66.7 | 15.9 | 11-28 |
| 10 | 1 | 50.0 | 22.0 | ..... | 1 | 100.0 | 14.5 | ..... |
| 11 | 0 | .... | .... | ..... | 0 | .... | .... | ..... |
| Totals | 96 | 75.6 | 20.8 | 7.5-35 | 78 | 83.0 | 18.1 | 6.5-33 |

* The percentage following the number of cases is the percentage of the total number of cases studied in that decade.

Kansas, found an average incidence of 18.4% for both sexes, but Hazard and Kaufman,[6] in Cleveland, obtained an incidence of 8.2% in males and 7.8% in females. Only two of the glands included in the present group of 47 (31 males, 16 females) were without nodules or other primary thyroid lesions. One weighed 40.5 gm. in a 52-year-old man dying with cirrhosis of the liver; the other weighed 66.5 gm. and was heavily infiltrated with atypical lymphocytes in a patient who died of chronic lymphocytic leukemia.

Also seen in Tables 1, 2, 4, and 5 is the tendency for the thyroid gland to be heaviest in the early and middle decades and to become lighter with advanced years. This finding is similar to that of other studies, such as those of Nolan[5] and Rice,† in which weights by decades are published. This finding is interpreted as reflecting physiologic atrophy.

An attempt to correlate thyroid gland weight with body weight, body height, or body surface gave such poor results in this study that the idea was abandoned. Marine ‡ determined the normal thyroid gland weight to be up to 0.35 gm. per kilogram of the body weight but noted that the weight could be greater in women and much greater in children and infants. Wegelin,§ citing Huschke, gave the normal weight for the thyroid gland in newborn infants of 1/423 of the birth weight and 1/1,800 of the adult weight.

*Distribution of Thyroid Nodules.*—In order to determine the distribution of nodules of the various types, the thyroid gland was divided into five arbitrary areas as follows:

† References 2 and 19.

‡ Marine,[20] p. 803, as cited by Nolan.[5]

§ Wegelin,[21] Vol. 8, p. 16.

TABLE 5.—*Thyroid Glands Weighing Over Thirty-Five Grams, Based on Forty-Seven Cases*

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Cases | | Av. Wt., | Extremes of Wt., | Cases | | Av. Wt., | Extremes of Wt., |
| Decade | No. | %* | Gm. | Gm. | No. | %* | Gm. | Gm. |
| 2 | 0 | .... | .... | ..... | 0 | .... | .... | ..... |
| 3 | 2 | 28.6 | 42.5 | 36-49 | 1 | 12.5 | 36.5 | ..... |
| 4 | 0 | .... | .... | ..... | 0 | .... | .... | ..... |
| 5 | 1 | 6.3 | 107.0 | ..... | 1 | 11.1 | 91.0 | ..... |
| 6 | 2 | 22.2 | 43.5 | 40.5-46.5 | 0 | .... | .... | ..... |
| 7 | 8 | 28.6 | 51.1 | 38-74 | 4 | 23.5 | 45.1 | 35.5-61 |
| 8 | 13 | 32.5 | 46.9 | 36-74 | 7 | 26.9 | 58.7 | 39.5-120 |
| 9 | 4 | 23.5 | 46.8 | 40-53 | 2 | 33.3 | 46.5 | 41-52 |
| 10 | 1 | 50.0 | 90.0 | ..... | 0 | .... | .... | .... |
| 11 | 0 | .... | .... | ..... | 1 | 100.0 | 47.0 | ..... |
| Totals | 31 | 24.4 | 61.1 | 36-90 | 16 | 17.0 | 54.1 | 35.5-120 |

* The percentage following the number of cases is the percentage of the total number of cases studied in that decade.

superior half of the right lobe, inferior half of the right lobe, isthmus, superior half of the left lobe, and inferior half of the left lobe. The number of thyroid glands involved with nodules in each division was recorded. A similar break-down of nodules by location in the thyroid gland was not found in the literature. The incidence of thyroid glands containing nodules in each of the five divisions was roughly proportional to the weight of each division. The inferior half of each lobe was the portion most frequently involved by nodules. As would be expected, the incidence of thyroid glands containing nodules in the isthmus was lowest. The incidence of thyroid glands containing nodules 10 mm. or larger in diameter was also recorded and paralleled the incidence of all thyroid glands containing nodules as to location. The recording of nodules by size has clinical importance, since 10 mm. is the apparent lower limit of clinical palpability. This standard was used by Hazard and Kaufman [6] and was borne out by the search of the clinical records in the cases used in this report. The correlation of clinical and necropsy findings with regard to size of thyroid nodules is discussed below.

Although follicular nodules constituted the bulk of the nodules found, the distribution of fetal nodules was comparable. The few embryonal and oxyphil cell nodules observed prevented similar comparisons.

The term "oxyphil cell nodule" is used in place of "Hürthle cell nodule" because of the preference for a meaningful descriptive term rather than for an incorrect term that has been retained merely because of common usage in the past. In their article on "Hürthle" cell tumor of the thyroid gland, Chesky, Dreese, and Hellwig [22] give an excellent historical review of how this cell was named. They point out that, in 1877 and in 1882, an interfollicular oxyphilic cell was first described in the thyroid gland of laboratory animals of unspecified species by Baber.‖ In 1894 a similar cell was described in the thyroid gland of the dog by Hürthle.¶ In 1907 human tumors composed of this cell were described by Langhans # and by Getzowa.* Langhans called the tumors "small alveolar, large-celled struma," but Getzowa felt that they originated in cell rests of the ultimobranchial bodies and called them "struma postbranchialis." In 1923 Williamson and Pearse [28] doubted the existence of interfollicular epithelial cells in the human thyroid gland. In 1926 Wegelin † denied the possibility that "Hürthle cells" came from cell rests of the ultimobranchial bodies and felt that they were functional variants of thyroid epithelium. Since 1928, when Ewing [29] used the term "Hürthle cell tumor" in the caption of his Figure 492a in describing a tumor composed of large oxyphilic cells, the term has enjoyed wide usage. In 1929 Rienhoff [30] conclusively proved with wax reconstructions of the thyroid gland that interfollicular cells do not exist in the human thyroid gland. In 1938 Wilensky and Kaufman [31] doubted the anatomic specificity of the "Hürthle cell" because all grades of transition from normal thyroid epithelial cells to the large acidophilic cell could be found. In 1948 Willis [32] felt that the term "Hürthle cell" was unfortunate. After reviewing all of this evidence, Chesky, Dreese, and Hellwig [22] conclude that the term is too well established to discontinue. I cannot agree with this conclusion and should like to join Mulligan [33] in advocating the term "oxyphil cell" as being more descriptive and meaningful in designating the abnormal cell which originates in alterative fashion from the normal thyroid parenchymal cell and is not identical with the normal interfollicular cell, which Baber and Hürthle described for lower mammals. Worthy of consideration is the work of Hamperl [34] with histochemical stains for the identification of "onkocytes" in vari-

‖ References 23 and 24, cited by Chesky, Dreese, and Hellwig.[22]

¶ Hürthle,[25] cited by Chesky, Dreese, and Hellwig.[22]

# Langhans,[26] cited by Chesky, Dreese, and Hellwig.[22]

* Getzowa,[27] cited by Chesky, Dreese, and Hellwig.[22]

† Wegelin,[21] pp. 191-193.

TABLE 6.—*Incidence of Thyroid Glands with Nodules by Decades, Based on Seventy-Eight Nodular Glands in Males and Sixty-Three in Females*

| Decade | Cases, No. | Nodules, 1 to 9 Mm. No. | Nodules, 1 to 9 Mm. % | Nodules, 10 Mm. or Larger No. | Nodules, 10 Mm. or Larger % |
|---|---|---|---|---|---|
| 2..... | 1 | 1 | 100.0 | 0 | 0.0 |
| 3..... | 7 | 5 | 71.4 | 2 | 28.6 |
| 4..... | 4 | 4 | 100.0 | 0 | 0.0 |
| 5..... | 15 | 12 | 80.0 | 3 | 20.0 |
| 6..... | 9 | 5 | 55.6 | 4 | 44.4 |
| 7..... | 32 | 20 | 62.5 | 12 | 37.5 |
| 8..... | 52 | 22 | 42.3 | 30 | 57.7 |
| 9..... | 17 | 7 | 41.3 | 10 | 58.7 |
| 10..... | 3 | 1 | 33.3 | 2 | 66.7 |
| 11..... | 1 | 1 | 100.0 | 0 | 0.0 |
| Totals | 141 | 78 | 55.3 | 63 | 44.7 |

ous organs, including the thyroid gland. His methods indicate that the oxyphil (Hürthle) cell of the thyroid gland in man is identical with "onkocytes" found in the salivary glands and elsewhere, in that these originate by pathologic alteration of normal parenchymal cells. His findings support the opinion of many that the "onkocyte," wherever found, is a degenerative product of parenchymal cells.

The incidence of thyroid glands with nodules is shown in Table 6. In 78 thyroid glands from males and 63 from females nodules of various types and sizes were found. Of these glands, 57.0% in males and 54.0% in females contained only nodules from 1 to 9 mm. in greatest dimension, while 46.8% of thyroid glands in males and 49.2% of those in females contained nodules 10 mm. or more in greatest dimension. In the latter groups most of the glands also contained smaller nodules. Of the 221 glands examined, 64.6% contained nodules of some type. Rice [19] also lists the incidence of thyroid glands with nodules by decades and cites a similar study in Switzerland referred to by Wegelin.‡ In all of these studies the steady increase in incidence of thyroid glands with nodules as age advances is apparent.

TABLE 7.—*Comparison of Incidence of Thyroid Glands with Nodules*

| Author | Cases, No. | Per Cent with Nodules |
|---|---|---|
| Rice,[2] Minnesota, 1932 (surgical and autopsy) | 555 | 56.95 |
| Hellwig,[4] Kansas, 1935 (autopsy) | 100 | 38.2 |
| Nolan,[5] Minnesota, 1938 (autopsy) | 725 | 26.3 |
| Schlesinger, Gargill, and Saxe,[7] Massachusetts, 1938 (autopsy; nodules 10 mm. or larger) | 1,373 | 8.2 |
| Coffey, Amoroso, and Mazzara,[8] Pennsylvania, 1951 (surgical) | 431 | 71.8 |
| Hull, Colorado, 1954 (autopsy) | 221 | 64.6 |

In Table 7 a comparison is made with figures from other studies. The incidence of thyroid glands with nodules is higher in Colorado than in any other area reported upon in the United States. The series of Coffey, Amoroso, and Mazzara [8] pertains only to surgical material and would concern only clinically abnormal glands. The series of Schlesinger, Gargill, and Saxe [7] reports only nodules larger than 10 mm. and reflects the effect of a seacoast location or nonendemic goiter area. The listing by decades shows also that nodules are uncommon in the second and third decades, especially those 10 mm. in diameter or larger. The largest nodule encountered was an 80 mm. involuted follicular mass, extending from the lower pole of one thyroid gland to the suprasternal notch.

TABLE 8.—*Thyroid Glands Containing Various Types of Nodules*

| Types of Nodules | No. | % |
|---|---|---|
| Thyroid glands with nodules examined | 142 | .... |
| Thyroid glands containing only follicular nodules | 92 | 64.8 |
| Thyroid glands containing a mixture of follicular plus fetal, embryonal, or oxyphil nodules | 42 | 29.6 |
| Thyroid glands containing multiple fetal, embryonal, or oxyphil nodules | 14 | 9.9 |
| Thyroid glands containing only fetal, embryonal, or oxyphil nodules | 6 | 4.2 |

In Table 8 the number of thyroid glands involved by different types of nodules are recorded. Follicular nodules are commonest, being the only type found in 92 of the 142 glands containing nodules. This incidence of 64.8% is compared to 82.5% found by Rice.[2] In 42, or 29.6%, of the glands containing nodules, follicular nodules were found in combination with fetal, embryonal, or oxyphil cell nodules. Multiple fetal, embryonal, or oxyphil nodules were found in

‡ Wegelin,[21] Vol. 8, p. 16.

14, or 9.9%, of thyroid glands with nodules. A report of the incidence of multiple fetal, oxyphil, or embryonal nodules in the thyroid gland was not found in the literature. Marclay [35] reported a case in which multiple fetal adenomas in a surgically removed thyroid gland was thought to be rare. Only 6, or 4.2%, of the glands with nodules contained only fetal, embryonal, or oxyphil nodules. Rice [2] reported that 25.5% of thyroid nodules are of fetal type and that 9.3% of solitary nodules are of fetal type.

The relationship of thyroid nodules to the cause of death is given in Table 9. The highest percentage of thyroid glands with nodules is encountered in the group in which arteriosclerosis was the major cause of death. This included such causes as arteriosclerotic heart disease with cardiac failure or coronary thrombosis and cerebrovascular accidents due to arteriosclerosis of cerebral arteries. Arteriosclerosis frequently was advanced in the patients dying with cancer and acute infection, though it was not a direct cause of death. These groups also had a high incidence of thyroid glands with nodules. The patients dying from other types of cardiac disease included only those with rheumatic or congenital heart disease and were of much younger average age. Patients dying of kidney disease included young persons with glomerulonephritis and older men with prostatic obstruction and chronic pyelonephritis. Nolan [5] listed the cause of death in his cases, but did not note the number of thyroid glands with nodules in each group. The high incidence of nodules in patients with advanced arteriosclerosis suggests that nodules in the thyroid gland are a result of regressive change.

*Solitary Nodules.*—Because of the apparent serious implications of a solitary nodule in the thyroid gland, this problem has been dealt with separately. In Table 10 the solitary nodules found in 141 thyroid glands with nodules are listed by decades, totals, and percentages. In 19 glands the solitary nodules were from 1 to 9 mm. in diameter; in 5 glands, 10 mm. or larger. This gives a total of 24 glands with solitary nodules, or an inci-

TABLE 9.—*Thyroid Glands Containing Nodules as Related to Cause of Death*

| Cause of Death | Without Nodules | | With Nodules | | With Nodules, 10 Mm. or Larger | | Total |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. |
| Cancer | 21 | 35.0 | 39 | 65.0 | 16 | 26.7 | 60 |
| Arteriosclerosis | 8 | 16.7 | 40 | 83.3 | 21 | 43.8 | 48 |
| Acute infection | 8 | 23.5 | 26 | 76.5 | 15 | 44.1 | 34 |
| Chronic infection | 11 | 57.9 | 8 | 42.1 | 4 | 10.5 | 19 |
| Nephritis | 7 | 50.0 | 7 | 50.0 | 5 | 35.7 | 14 |
| Cardiac disease | 8 | 61.5 | 5 | 38.5 | 2 | 15.4 | 13 |
| Accident | 5 | 71.4 | 2 | 28.6 | 1 | 14.3 | 7 |
| Gastric ulcer | 3 | 42.9 | 4 | 57.1 | 0 | 0.0 | 7 |
| Diabetes | 1 | 33.3 | 2 | 66.7 | 0 | 0.0 | 3 |
| Cirrhosis | 2 | 100.0 | 0 | 0.0 | 0 | 0.0 | 2 |
| Miscellaneous | 5 | 35.7 | 9 | 64.3 | 0 | 0.0 | 14 |
| Totals | 79 | | 142 | | 64 | | 221 |

TABLE 10.—*Incidence of Thyroid Glands with Solitary Nodules by Decades, Based on Seventy-Eight Nodular Glands in Males and Sixty-Three in Females*

| Decade | Cases, No. | Glands with a Nodule 1-9 Mm. | Glands with a Nodule 10 Mm. or Larger | Glands with a Nodule 10 Mm. or Larger and Smaller Nodules |
|---|---|---|---|---|
| 2 | 1 | 1 | 0 | 0 |
| 3 | 7 | 0 | 1 | 1 |
| 4 | 4 | 3 | 0 | 0 |
| 5 | 15 | 5 | 2 | 0 |
| 6 | 9 | 3 | 0 | 2 |
| 7 | 32 | 3 | 0 | 7 |
| 8 | 52 | 2 | 1 | 13 |
| 9 | 17 | 1 | 1 | 6 |
| 10 | 3 | 1 | 0 | 0 |
| 11 | 1 | 0 | 0 | 0 |
| Totals | 141 | 19 | 5 | 29 |
| Per cent | | 13.5 | 3.5 | 20.6 |

dence of 17.0% of the thyroid glands with nodules. The incidence for the entire 221 glands examined was 10.9%. Three of the 19 solitary nodules from 1 to 9 mm. in diameter were fetal nodules; one of the nodules 10 mm. or larger was fetal. A separate category of a solitary large nodule associated with one or more small nodules is also presented in Table 10. These thyroid glands would probably be reported clinically as containing solitary nodules. In this group of 29 thyroid glands, 8 of the large solitary nodules were fetal, 2 were oxyphil, and the remaining 19 were follicular. The largest solitary nodule encountered was a fetal nodule 70 mm. in diameter.

TABLE 11.—*Comparison of Incidence of Thyroid Glands Containing Solitary Nodules*

| Author | Number of Thyroids Examined | Per Cent Thyroids with Solitary Nodules |
|---|---|---|
| Rice,[2] Minnesota, 1932 (surgical and autopsy) | 555 | 20.7 |
| Coffey, Amoroso, and Mazzara,[5] Pennsylvania, 1950 (surgical) | 431 | 25.8 |
| Hazard and Kaufman,[6] Ohio, 1952 (autopsy) | 429 | 12.0 |
| Beal, Scholnick, and Stevens,[10] California, 1952 (surgical) | 200 | 32.0 |
| Hull, Colorado, 1954 (autopsy) | 221 | 10.9 |

All three of the incidental papillary carcinomas of the thyroid discovered in this study were found in this group of 29 glands with a solitary nodule 10 mm. or larger and additional smaller nodules. In all three cases, the accompanying large solitary nodule was either of fetal or oxyphil cell type.

Much has been written concerning solitary nodules in the thyroid gland. A comparison of the incidence in this study and in some of the previous studies is made in Table 11. In the group reported by Hazard and Kaufman,[6] 12.0% of all thyroid glands examined contained solitary nodules, as compared to 10.9% of all glands in the present study. In Table 11, the other groups summarized are based on surgical material, or a combined surgical and autopsy group, and have a correspondingly larger incidence of solitary nodules due to clinical selection. Hazard and Kaufman[6] also report that, of their solitary nodules, only 2% were larger than 10 mm. Of the 24 solitary nodules found in this study, 20% were 10 mm. in diameter or larger. Of the 111 solitary nodules reported by Coffey, Amoroso, and Mazzara,[5] 52 were follicular nodules, 17 were fetal, and 24 were carcinoma of various types. Since he studied only surgically removed tissue, the figures were heavily weighted on the side of cancer. Rice[2] also found that 62.79% of solitary nodules were of follicular type, with 9.3% of fetal type. These figures compare to 83.3% and 16.7% in my study.

An increasing number of extrinsic and intrinsic factors are being shown to affect the histology and function of the thyroid gland. Iodine, fluorine, calcium, arsenic, cyanates, vitamins A and D, elements in edible plants of the Brassicae family, and some factor in liver have all been shown to be influential in thyroid gland function. The data implicating these factors are reviewed by Fertman and Curtis.[36] Money, Kirschner, Kraintz, Merrill, and Rawson[37] have shown that, intrinsically, the thyroid-stimulating hormone of the anterior pituitary gland is the most important single factor, but secretions of the adrenal gland and gonads also affect thyroid function. Van Dyke[38] in his experiments with rats has shown that age is important in the incidence and numbers of nodules developed in the thyroid gland. Aged rats (over 300 days) developed more nodules and increasing numbers of nodules spontaneously than did young rats (under 100 days old). The old rats also developed more nodules after stimulation with goitrogenic drugs than did the young rats. Money and Rawson[39] have produced localized benign tumors in rats by prolonged feeding of thiouracil, as have many others. Morris, Dalton, and Green[40] produced numerous cancers of the thyroid gland in one strain of mice by a similar feeding program, but could rarely produce cancer of the thyroid gland in another strain of mice on the same regimen. This suggests a familial variation in reaction to stimulation or depression of thyroid activity. Goldberg and Chaikoff[41] produced thy-

roid adenomas in rats with propylthiouracil feeding, and cancer in the thyroid gland in rats with radioactive iodine treatment. Greenwald [42] suggests that intoxication or infection may play as important a role in goiter development as iodine deficiencies. The balance between these many factors, rather than the absence or excessive presence of any one of them, appears to be most important in determining the type of reaction which the thyroid gland will display. Though the ultimobranchial bodies are implicated in many thyroid gland nodules in lower animals, Maximow and Bloom [43] doubt their importance in the human thyroid gland. Unanswered is the reason for the variation between focal and diffuse response to stimulation or depression shown in different thyroid glands. DeCourcy and DeCourcy [44] suggest that nodules in the thyroid gland represent a form of irregular exhaustion atrophy following alternate hyperplasia and involution. I should like to suggest that repeated cycles of focal hyperplasia and involution lead to a form of retroplasia of normal epithelium. Follicular, fetal, and embryonal types of thyroid nodules probably represent forms of decreasing functional capacity of the normal thyroid epithelial cell, with the oxyphil cell nodule representing the ultimate in physiologic exhaustion.

*Primary Carcinoma of the Thyroid Gland.*—Data concerning the three incidental primary carcinomas of the thyroid gland in this series are as follows:

Case 52-239 was a 71-year-old man with a 20 gm. thyroid gland. The carcinoma was 30 mm. in greatest diameter, occupied the entire right lobe of the gland, and was accompanied by a fetal nodule in the left lobe of the thyroid gland.

Case 53-97 was a 75-year-old man with a 56 gm. thyroid gland. The carcinoma was less than 1 mm. in diameter, was located in the inferior portion of the left lobe, and was accompanied by multiple follicular and fetal nodules.

Case 53-222 was a 75-year-old man with a 23 gm. thyroid gland. The carcinoma was 4 mm. in diameter, was located in the inferior portion of the left lobe, and was accompanied by multiple follicular and oxyphil cell nodules.

These three cases in which an incidental papillary carcinoma of the thyroid gland was found represent an incidence of 1.4% of the 221 thyroid glands examined. Photomicrographs of these three carcinomas are shown in Figures 5, 6, and 7. All were found in men in the eighth decade, two in multinodular thyroid glands of normal size and one in an enlarged multinodular gland. In Case 52-239 a second focus of tumor was found inferior to the lower pole, on the same side as the carcinoma. This may have been a lymph node, but insufficient lymphoid structure remained for definite identification. No other primary or metastatic foci were found. In the other two cases multiple follicular nodules were present. In Case 53-222 a single 12 mm. oxyphil nodule was present in the same lobe with the carcinoma, but in Case 53-97 six fetal nodules were scattered throughout the thyroid gland. Case 53-97 might be termed a small papillary adenoma by some,[45] but is probably better qualified for the classification of nonencapsulated sclerosing tumor of Hazard, Crile, and Dempsey,[12] who concluded that this lesion represents a low-grade primary thyroid carcinoma, usually of papillary type. In all three of my cases, the carcinoma occurred in glands the seat of benign nodules. No evidence was obtained that these three carcinomas developed in a previously benign nodule of any type. All three were regarded as carcinoma originating *de novo* and were associated with benign nodules of types usually designated adenoma. One of the cases in the present study, Case 52-239, could possibly be considered clinically detectable, but the patient had been well until he came to the hospital with an acute myocardial infarction. His tumor was not detected during routine examination on admission. The other two carcinomas were too small to be palpated by physical examination.

Hendrick and Reed [46] estimated the incidence of carcinoma of the thyroid gland at 0.5 to 1.0% in the general population, with

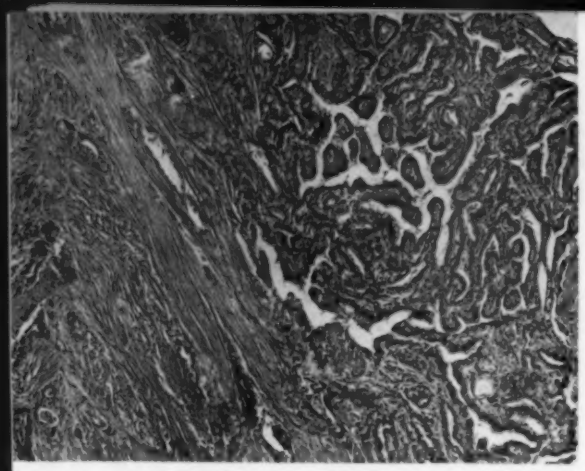
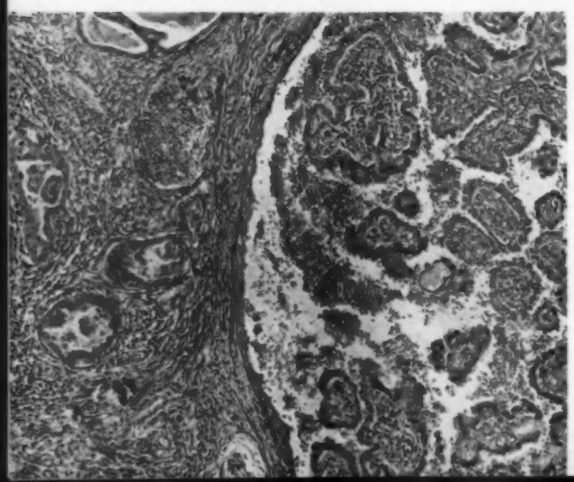


Fig. 5.—Papillary carcinoma in Case 52-239; reduced ¼ from mag. × 50.

Fig. 6.—Papillary carcinoma in Case 53-97; reduced ¼ from mag. × 85.

an incidence of 1.3 to 5.5% in tissue obtained at thyroidectomy. A comparison of the incidence of carcinoma in nodular goiters and in solitary thyroid nodules, as found by several investigators, is given in Table 12. All of these are taken from surgical series and show considerable variation in various portions of the country.

Fig. 7.—Papillary carcinoma in Case 53-222; reduced ¼ from mag. × 50.



Three primary carcinomas in my 221 cases are surprising in view of the figures reported by Vander Laan.[9] He found only five primary carcinomas of the thyroid gland in 18,668 autopsies in three Boston hospitals. However, he reviewed only autopsy protocols, and I doubt that his routine material represented as careful examination of the thyroid gland as was undertaken in my study. He also cites the series of Ophuls,[53] in which no primary thyroid carcinomas were found in 3000 autopsies, but the same comment is applicable here concerning the care in examination of the thyroid gland at autopsy. Schlesinger, Gargill, and Saxe[7] found six cases of unsuspected primary carcinoma in 1,373 thyroid glands examined at necropsy, but their figures are also taken from routine autopsy protocols. Queen,§ in Oregon, has been conducting a study of the thyroid gland similar to mine. He has found 20 unsuspected primary carcinomas in 1,217 thyroid glands, or an incidence of 1.6%.

Much has been written concerning carcinoma of the thyroid gland in nodular goiter

TABLE 12.—*Comparison of Incidence of Carcinoma in Nodular Goiters and in Solitary Thyroid Nodules Removed at Surgery*

| Author | % in Nodular Goiter | % as Solitary Nodules |
|---|---|---|
| Schlesinger, Gargill, and Saxe,[7] 1938.. | 4.5 | .... |
| Ward,[47] 1944 ..... | 4.8 | .... |
| Crile and Dempsey,[48] 1949..... | 5.6 | 24.5 |
| Cope, Dobyns, Hamlin, and Hopkirk,[15] 1949 ..... | 10.1 | 19.0 |
| Cole, Majarakis, and Slaughter,[14] 1949 ..... | 17.1 | 24.4 |
| Crotti,[16] 1950 ..... | 3.1 | 3.2 |
| Beahrs, Pemberton, and Black,[17] 1951 | 7.5 | .... |
| Hendrick and Reed,[46] 1951..... | 8.2 | 17.8 |
| Fisher and Fisher,[49] 1951..... | 3.3 | .... |
| Lahey and Hare,[50] 1951..... | 0.62 | 10.04 |
| Watt and Foushee,[51] 1951..... | 1.7 | 2.5 |
| Beal, Scholnick, and Stevens,[10] 1952.. | 10.8 | 17.2 |
| Crile,[52] 1953 ..... | 5.0 | .... |

§ Queen, F.: Personal communication to Dr. James B. McNaught in Denver. Department of Pathology, University of Oregon School of Medicine, April, 1954.

as compared to carcinoma in the solitary thyroid nodule. Graham,[13] in 1924, stated that 90% of all thyroid cancers developed in previously benign adenomas. This remained the accepted figure for many years. In 1930 Ward and Carr || reported 36 carcinomas in the thyroid gland and concluded that 94% originated in adenomas. In 1944 Ward [47] reported on 68 thyroid carcinomas and felt that half were carcinoma from the onset and that his 1930 figures were unjustified. He also noted that, whereas women more frequently have a nodular goiter than men, the incidence of carcinoma in nodular goiters is higher in men. In 1949 Crile and Dempsey [48] stated, "It is reasonable to suppose that many, if not most, carcinomas of the thyroid are carcinomas from the beginning and arise from the parenchyma of the thyroid as do benign adenomas. There is little proof that a benign tumor of the thyroid becomes malignant oftener than the goitrous thyroid parenchyma." In 1951 Lahey and Hare [50] stated, however, that it was "generally accepted that the origin of cancer of the thyroid is, for practical purposes, in the preexisting adenoma of the gland." In 1952 Meissner and McManus [55] stated that evidence "suggests that perhaps most papillary carcinomas . . . are malignant from their inception and do not arise from a preexisting benign phase." In 1953 Swinton [56] stated that more recent data presented by pathologists suggested that not all thyroid cancer arises from a preexisting benign thyroid tumor and that some nodules may be malignant from their onset. Finally, in 1953, Crile [52] stated that "it appears that most thyroid carcinomas are malignant from the first and that the transition of a benign adenoma to a . . . cancer is relatively rare, . . ." Thus, during the past 30 years, a slow evolution of thought has taken place. I subscribe to Crile's statement that carcinomas of the thyroid gland are usually malignant from inception.

One of the most troublesome problems in the analysis of the literature on the thyroid gland in an effort to determine the statistical evidence for the origin of carcinoma of the thyroid gland in solitary adenoma has been the lack of proof for benign adenoma preceding carcinoma. The three points which must be considered are as follows: (1) whether a solitary nodule is the primary carcinoma, (2) whether a solitary nodule represents a primary carcinoma developing by canceration of an adenoma, or (3) whether a nodule of primary carcinoma coexists in a gland containing one or more adenomas. These are important points which should be clarified in future studies of this type. Such careful differentiation will continue to correct a false impression, now 30 years old, and will eventually provide adequate data for determining which carcinomas of the thyroid gland develop as primary carcinomas and which develop by malignant change in a previously benign adenoma.

*Correlation of Clinical and Necropsy Findings.*—In spite of the fact that most of the patients were examined by a medical student, an intern, and a resident, only 7 of 43 thyroid glands weighing more than 35 gm. and 7 of 64 thyroid glands containing nodules 10 mm. or more in diameter were felt during routine physical examination. Schlesinger, Gargill, and Saxe [7] felt that nodules in the thyroid gland 10 mm. in diameter or larger should be considered significant, but also found that most of these nodules found at autopsy had not been suspected during antemortem examinations. The most glaring errors in this study consisted of calling normal a 107 gm. partially calcified gland with nodules up to 20 mm. in diameter and a 101 gm. gland with an 80 mm. mass extending into the suprasternal notch. The first case was in a 96-lb. (43.5-kg.) male and the second in a 114-lb. (51.7 kg.) female. Obviously these examinations were not done at all or were very hasty and inadequate. Such examples serve to point out that the thyroid gland is probably often given a very cursory check during physical examination and many significant lesions are missed. In order to increase the detection of thyroid lesions, the thyroid gland should be examined by the method advocated by Lahey and Hare [50] and

|| Reference 54, as cited by Ward.[47]

emphasized by Swinton.[56] This method is illustrated in Figure 8. This type of examination cannot be carried out without having specifically in mind the detection of lesions in the thyroid gland.

Of the seven nodules found and recorded during the clinical examination of the 221 patients in this study, four were only 15 mm. in diameter and were accurately located. The remaining three nodules recorded ranged up to 80 mm. in diameter.

*The Delphian Node.*—To quote from Cope, Dobyns, Hamlin, and Hopkirk,[15] "The Delphian node, named after the oracle of Delphi, lies in the midline of the neck just above the upper border of the thyroid isthmus and in front of the middle cricothyroid ligament." They state that this lymph node, or group of lymph nodes, is almost always present and found them in 94% of 152 surgical operations on the thyroid gland. They attached importance to this finding because these lymph nodes are involved early when surgical pathologic lesions, especially carcinoma, affect the thyroid gland. The diagram in Figure 9 shows the location of this group of lymph nodes. A microscopic group of lymph nodes closely associated with the superior edge of the isthmus is shown in Figure 10. In my series the lymph node, or nodes, were found in 120 cases, or 54.3%. One to five lymph nodes were found in each case. In 78 cases, the lymph nodes were found by gross inspection of the fibrous connective and fat tissue overlying the superior portion of the isthmus and the trachea immediately superior to the isthmus. After these were dissected free, they were all confirmed microscopically. In 22 of these 78 cases, additional lymph nodes were found microscopically by routine sectioning of all of the superior half of the isthmus and its associated fibrous connective tissue. In 42 cases, the lymph nodes were found only by microscopic examination of the fibrous connective tissue, fat, and superior half of the thyroid isthmus. The success in finding this structure depends largely on the diligence applied to the search.

Fig. 8.—Left, method of displacing the thyroid gland from its bed. Right, method for palpating the gland with the ball of the opposite thumb.

Adapted from Lahey, F. H., and Hare, H. F.: Malignancy in Adenomas of the Thyroid, J. A. M. A. **145**:689-695, 1951.

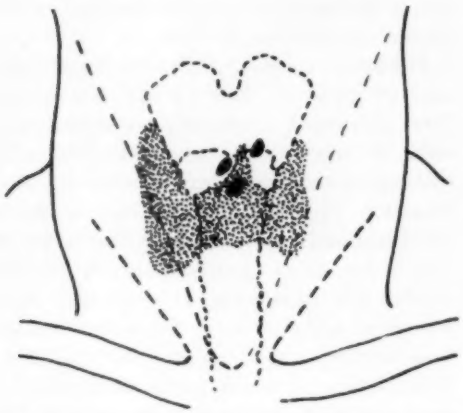


Fig. 9.—Diagram showing the location of the Delphian node. Lymph nodes are located in the fibrous connective tissue and fat associated with the superior half of the isthmus of the thyroid gland, and the trachea superior to the isthmus.

Adapted from Cope, O.; Dobyns, B. M.; Hamlin, E. Jr., and Hopkirk, J.: What Thyroid Nodules Are to Be Feared?, Tr. Am. Goiter A., pp. 256-266, 1949.

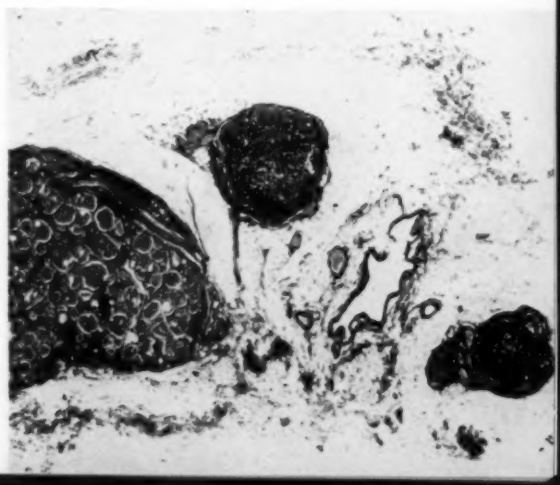
Gross discovery could be expected to be greater in the case of diseased thyroid glands exposed at surgery, as found by Cope, Dobyns, Hamlin, and Hopkirk,[15] than in those found at autopsy, as in the present series in which the incidence was 54.3%. This discrepancy between the high figure given by Cope and co-workers [15] and that of my series is easily explained by the rigorous selection of autopsy cases for their freedom from clinical thyroid disease. The only abnormal lymph nodes found in this series were those involved in leukemic infiltrations and in Hodgkin's disease. Queen ¶ found the Delphian node in 61 cases, or an incidence of 4.8%.

*Squamous Metaplasia.*—Squamous metaplasia was found in 6 thyroid glands without nodules and in 15 thyroid glands containing nodules. An example of this lesion is shown in Figure 11. This type of change was commonest in the advanced age groups. Of additional interest is the occurrence of metaplasia in 11 of the 48 thyroid glands from persons dying with primary arteriosclerotic disease and only twice in the 59 thyroid glands from patients dying of cancer. None of the areas of metaplasia was found within nodules. Klinck and Menk [57] reported on 41 cases of metaplasia in the thyroid gland, in which 16 were in cases of papillary carcinoma of the gland and 11 in nodular goiter. No metaplasia was found in relation to the three primary carcinomas recorded in my series. Bullock, Hummer, and Kahler [58] reported on 52 cases, reviewed the literature, and noted that squamous metaplasia had been found in the thyroid gland of the embryo, of cretins, of idiots, in areas of thyroiditis, in adenomas, and in association with involution. Jaffe [59] described four cases of squamous metaplasia in fibrosed glands and reported three cases of squamous cell carcinoma presumably starting in such tissue.

*Cancer Metastatic to the Thyroid Gland.*—In 10 of the thyroid glands taken from 59 persons dying of cancer, metastases of the same type of cancer were found. Of 45 cases of carcinoma, 3 showed metastases in the thyroid gland with primary sites in the stomach, prostate, and kidney. The metastasis from the renal carcinoma was found within a large fetal nodule. These lesions are illustrated in Figures 12, 13, and 14. Of seven cases of sarcoma, three showed metastases in the thyroid gland. All three were

¶ Queen, F.: Personal communication to Dr. James B. McNaught in Denver. Department of Pathology, University of Oregon School of Medicine, April, 1954.

Fig. 10.—Delphian lymph nodes found by microscopic examination of the superior half of the isthmus of the thyroid gland, and its associated fibrous connective tissue. The superior edge of the isthmus is to the left, the Delphian nodes in the upper center and to the right. Reduced ¼ from mag. × 15.



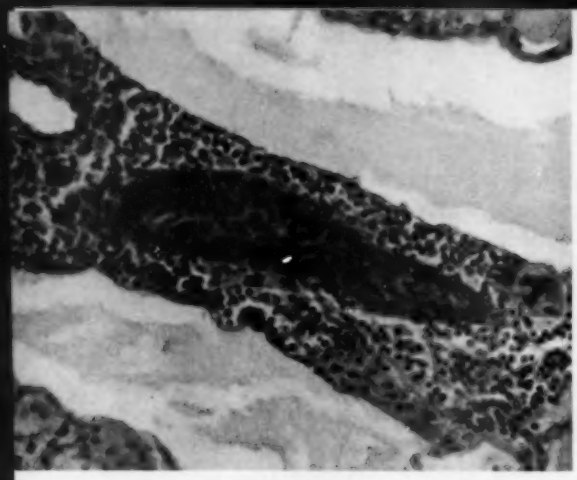
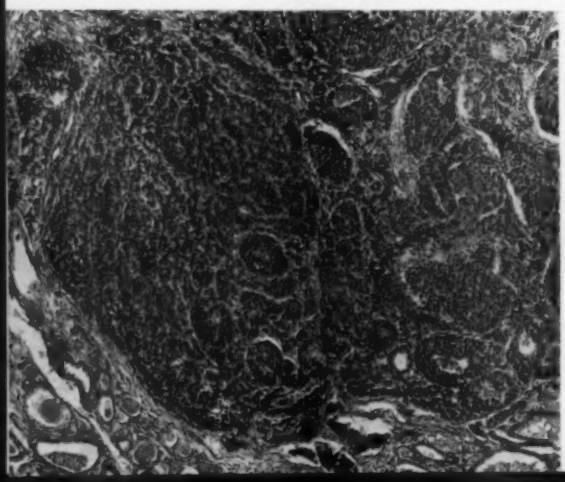


Fig. 11.—Squamous metaplasia in the thyroid gland; reduced ¼ from mag. × 165.

Fig. 12.—Carcinoma of the stomach metastatic to the thyroid gland; reduced ¼ from mag. × 50.

reticulum cell lymphosarcomas. Seven cases of leukemia were examined. Four, all lymphocytic, had infiltrations in the thyroid gland. One case of Hodgkin's disease was also examined and had infiltrations in the thyroid gland.

Fig. 13.—Carcinoma of the prostate metastatic to the thyroid gland; reduced ¼ from mag. × 50.



Metastatic tumors in the thyroid gland are well reviewed by Willis,# and his figures show carcinoma of the lung the commonest, with carcinoma of the breast, malignant melanoma, and carcinoma of the kidney next in order. He found an incidence of 5.2% thyroid gland involvement in his series of 170 autopsies on cancer patients. Rice [62] studied the thyroid glands at autopsy from 89 cancer patients and found 9 with metastases in the gland, or an incidence of 10.1%. Nolan [5] found two cases of malignant melanoma metastatic to the thyroid gland in 725 autopsies not selected for cancer. Queen * found metastatic cancer in 10 of the 1,217 thyroid glands which he has examined. Metastatic involvement of the thyroid gland in my series was 17.0% of all cases of cancer, or 11.6% of the carcinoma and sarcoma cases.

Metastases of other cancers to the thyroid gland may attain significant enlargement, as seen in one case in my series. Willis [61] reports that metastatic cancer in the thyroid gland can result in hyperthyroidism due to stimulation or hypothyroidism due to destruction of the gland.

*Miscellaneous Findings in the Thyroid Gland.*—A pyramidal lobe was found in 108, or 48.9%, of the thyroid glands examined. Beilby and McClintock [63] found a pyramidal lobe in 92.8% of 28 thyroid glands examined by them, but included fibrous connective tissue bands extending from the isthmus to the thyroid cartilage, 27% of which did not grossly contain thyroid tissue. According to the criteria in my study, the gross incidence of pyramidal lobes in their study was 73%. They cite a number of series from the literature, placing the incidence of the pyramidal lobe at from 33 to 90%. In the present series, gross and microscopic evidence of thyroid tissue was present in all pyramidal

# References 60 and 61.

* Queen, F.: Personal communication to Dr. James B. McNaught in Denver. Department of Pathology, University of Oregon School of Medicine, April, 1954.

lobes observed. The pyramidal lobes found showed all varieties of nodules, squamous metaplasia, and metastatic cancer, though in few cases, as would be expected from the minimal amount of thyroid tissue often present in this structure. Beilby and McClintock [63] attached surgical importance to the pyramidal tissue because of its implication in recurrent hyperthyroidism when left behind at surgery.

In eight cases accessory thyroid tissue was found inferior to the lower poles or to the isthmus. None of these accessory pieces of thyroid tissue exceeded 10 mm. in diameter, and most were closely connected to the thyroid gland by fibrous connective tissue bands. This finding is consistent with the type of non-neoplastic accessory thyroid tissue found by Warren and Feldman.[64]

In eight cases the isthmus of the thyroid gland was absent, or an incidence of 3.6%. Anderson [65] states that this is a rare anomaly, but gives no figures. Poate and Spencer [66] refer to 28 cases of hemiaplasia of the thyroid gland and its potential surgical importance; this anomaly was not encountered in my cases.

One or more parathyroid glands were found intimately associated with the thyroid gland in 33 cases. In 21 of these, the parathyroid gland was found buried in the thyroid capsule and was not seen during the original dissection. This type of location is shown in Figure 15. In 13 cases the parathyroid gland was buried within thyroid tissue and was not visible from the surface. This type of location is shown in Figure 16. One parathyroid gland of this latter group was located in the isthmus, one formed an asymptomatic adenoma 10 mm. in diameter, in two cases two parathyroid glands on the same side were buried within the thyroid tissue, and in two cases parathyroid glands were buried in the inferior portion of both lobes of the thyroid gland. Most of the parathyroid glands were found in the inferior half of the thyroid lobes. The surgical significance of the location of these glands within thyroid tissue is obvious. The policy of leaving the posterior portion of the thyroid gland during a subtotal

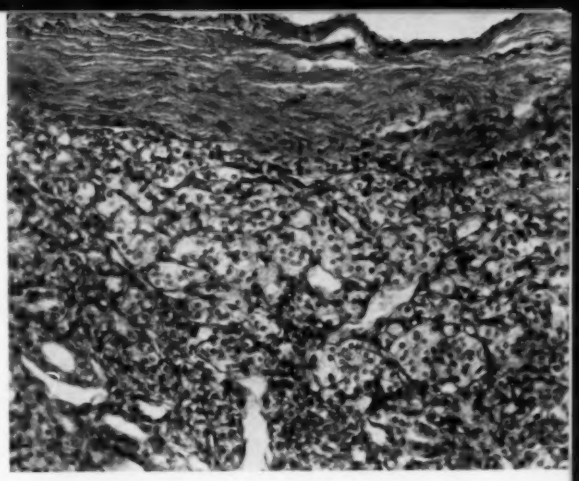


Fig. 14.—Carcinoma of the kidney metastatic to a fetal nodule in the thyroid gland. The metastatic tissue is directly below the fibrous capsule of the thyroid nodule; reduced ¼ from mag. × 165.

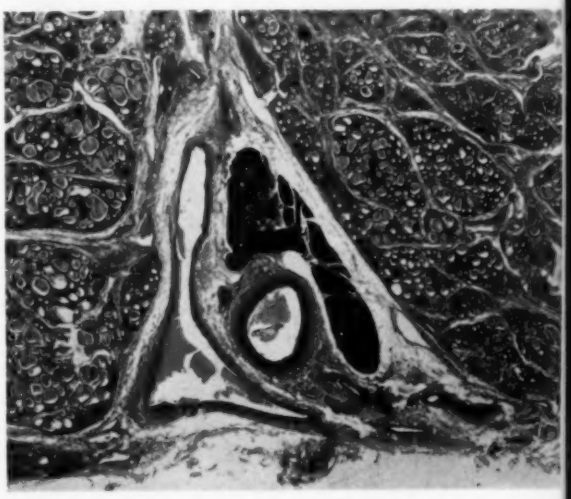
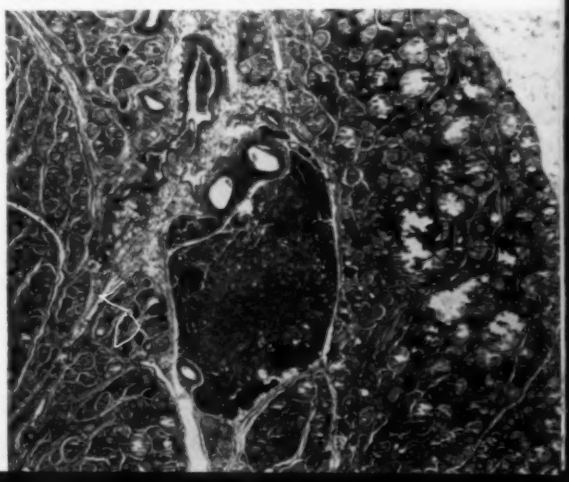


Fig. 15.—A parathyroid gland is present in the center of the photomicrograph surrounded by the fibrous connective tissue and vessels of the capsule of the thyroid gland; reduced ¼ from mag. × 15.

Fig. 16.—A parathyroid gland is present in the center of the photomicrograph, completely surrounded by thyroid tissue; reduced ¼ from mag. × 15.



thyroidectomy would have saved many of these parathyroid glands, but several would probably have been sacrificed, including the gland in the isthmus. In the case of total thyroidectomy all of these buried parathyroid glands would have been sacrificed, and in two cases in which parathyroid glands were buried in both lobes of the thyroid gland serious complications might have resulted. Had it become functional, finding the buried parathyroid adenoma in a multinodular thyroid gland could have presented an interesting surgical problem.

Thymic tissue was found in the superior portion of a lobe of one thyroid gland, and a small area of cartilage was found in the inferior portion of a lobe of another gland. In 48 cases fetal fat was found about parathyroid glands and in the fat tissue from the region of the isthmus. The presence of this type of tissue leads to the speculation that a hibernoma could possibly develop in this part of the body.

*Adenoma.*—The term adenoma has been deliberately avoided throughout the discussion. Warren and Meissner [45] set forth the criteria for differentiating between a nodule of a nodular goiter (adenomatous goiter nodule) and a true thyroid adenoma. These criteria are discussed individually in the light of the material examined in my series. "Adenomatous goiter nodules" are said to be multiple, and true thyroid adenomas, solitary. While it may be true that most "adenomatous goiter nodules" are multiple, a great many in my series were solitary, and most of the solitary nodules found were of the follicular type. This type is the one usually associated with nodular goiter and is not regarded as a true thyroid adenoma by most authorities, but, rather, it is thought to be an involutionary change in previously hyperplastic thyroid tissue. This point of view is adequately stated and discussed by Fisher and Fisher [49] and by Rice.[2] In my study, structures usually classified as adenomas, such as fetal, embryonal, and oxyphil nodules, were much commoner as multiple lesions or mixed with follicular nodules rather than occurring as solitary lesions.

Encapsulation as a criterion of the differentiation between the nodule of nodular goiter and true thyroid adenoma is often of no use. In the present study, follicular nodules with thick fibrous connective-tissue delimiting zones were encountered as often as fetal nodules with a smooth blending of abnormal and normal architecture. Variable and uniform structure were equally difficult to substantiate. A single pattern usually predominated, but instances of overlapping were numerous. Compression of adjacent gland tissue is common to both nodule and adenoma, and its absence is almost equally as common. If the follicular nodules cannot be considered expanding lesions, then the compression of adjacent thyroid tissue often seen about these structures must be due to shrinkage of the thyroid tissue while the nodule retains its original size or shrinks at a slower rate. This explanation of compression about follicular nodules has certain attractions, since it is known that the thyroid gland is largest and heaviest in the younger age groups, when nodules are relatively uncommon, and smaller and lighter in the older age groups, when nodules are more numerous.

Growth pattern was the criterion used and most depended upon in the analysis of the nodules found in my series. This served to differentiate follicular, fetal, embryonal, and oxyphil cell nodules, but no attempt was made to separate the true adenomas from the nonadenomatous nodules. Most nodules in the thyroid gland do not fulfill all criteria prescribed for their designation as adenomas. Statistical testing of all advocated criteria should be made by examining thoroughly a significant number of nodules of all types and by recording the presence or absence of the diagnostic characteristics to differentiate a nodule from an adenoma.

My concept of the histogenesis of nodules in the thyroid gland comes from an expansion of the data presented. Repeated stimulation and depression of thyroid function causes cycles of hyperplasia and involution. Gradual physiologic exhaustion leads to areas of permanent hyperinvolution and cellular

retroplasia. The cellular retroplasia may apply to single epithelial cells in a thyroid follicle, to the epithelial cells of an entire follicle, or to the epithelium of several follicles formed into a nodule. The oxyphil cell represents the end-stage of the retroplastic progression. The mechanism responsible for the focal response of the thyroid gland to stimulation or depression has not been determined. Single epithelial cells or small groups of epithelial cells showing retroplastic change are more numerous than large nodules composed of such retroplastic cells, forming follicles or solid cords. Under the influence of proper neoplastic stimulation, any of these retroplastic units may develop into true adenomas. Both nonadenomatous nodules and true adenomas may show growth patterns designated as follicular, fetal, embryonal, and oxyphil cell.

## SUMMARY AND CONCLUSIONS

During 1952 and 1953, a total of 221 thyroid glands was obtained at necropsy examinations performed in Colorado General Hospital. The critical analysis of these thyroid glands is the first study of this type in the Rocky Mountain area.

Normal thyroid glands had an average weight of 19.3 gm. in males and 17.7 gm. in females. The incidence of thyroid glands with nodules was 62.2% in males and 67.0% in females. Nodules were commonest in the older age groups. Solitary nodules were found in 17.0% of the 141 thyroid glands with nodules, or 10.9% of the total 221 thyroid glands examined, but none contained carcinoma. The three incidental primary carcinomas found represent an incidence of 1.4% and all were found in multinodular glands.

Only 17.5% of enlarged thyroid glands and 12.3% of glands containing nodules 10 mm. in diameter or larger were detected during routine physical examination.

The Delphian node was found in 54.3% of the cases. Squamous metaplasia was found in 9.5% of the thyroid glands examined, being twice as common in nodular as in nonnodular glands. Cancer metastatic to the thyroid gland was found in 16.9% of thyroid glands from patients dying with cancer. Pyramidal lobes were found in 48.9% of the thyroid glands examined. In 3.6% of cases accessory thyroid tissue was found closely associated with the inferior edge of the thyroid gland. In 3.6% of cases no thyroid tissue was found in the region of the isthmus. In 5.9% of cases parathyroid glands were found buried within thyroid tissue.

The criteria for differentiating between a nodule of nodular goiter and a true adenoma of the thyroid gland are reviewed and criticized.

This study was made under the supervision of Dr. Richard M. Mulligan, Professor of Pathology, as partial fulfillment of the requirements for the degree of Master of Science in Pathology.

Marguerite Jenks prepared the microscopic sections and Glenn Mills prepared the photographs.

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# Necrotizing Vascular Disease in Rat

## I. Observations on Pathogenesis

SIMON KOLETSKY, M.D., Cleveland

Over the past few years a variety of techniques was investigated in this laboratory in order to find a simple and effective method for the uniform rapid production, i. e., within a few days, of vascular necrosis in the rat. It was found that ligation of large branches of the renal arteries with resultant renal infarction was a very suitable procedure.

This technique was utilized to investigate the pathogenesis of necrotizing vascular disease. The origin of the latter is still uncertain. The results help to clarify several aspects of the problem, including the role of hypertension and uremia.

### METHOD

White male rats of the Wistar strain, weighing about 180 gm. each, were used. Renal infarction was produced as follows: (*a*) ligation of the anterior branch of the left renal artery and resection of the right kidney (15 rats); (*b*) ligation of the anterior branch of the left renal artery with the right kidney intact (15 rats), and (*c*) ligation of the anterior branch of the left renal artery and of the main right renal artery (15 rats). The vessels were tied with silk thread. Ligation of the anterior branch of the left renal artery produced infarction of the anterior half of this organ, while ligation of the right main renal artery caused an infarct of the entire kidney. All animals were killed from one to seven days after surgery.

There were 38 additional rats which had renal infarction by one of the above procedures. Of these, 6 were treated with antibiotics, 6 were treated with N,N-dibenzyl-$\beta$-chloroethylamine (Dibenamine), 6 were used to measure sodium space (with $Na^{24}$) on the third or fifth day after operation and then killed, and 20 had simultaneous arterial ligation and bilateral adrenalectomy. The animals which received antibiotics or Dibenamine were killed on the fourth to sixth day after surgery.

Antibiotic therapy consisted of 0.05 cc. of Combiotic (200,000 units of penicillin and 250 mg. of dihydrostreptomycin) given intramuscularly for two days prior to surgery and then daily thereafter. Dibenamine was given by stomach tube in doses of 10 mg/100 gm. of body weight (1.6 to 2.0 cc. of a 1% solution), starting the day before surgery and then daily thereafter.

Twenty sham-operated rats served as controls. These were subjected to the same surgical procedure as the experimental animals, except that the final step, i. e., renal artery ligation, was not carried out. The animals were killed within one week after operation.

Surgery was performed with clean technique, with the animal under sodium pentobarbital anesthesia, administered intraperitoneally. All procedures were completed in one stage. Diet consisted of chow and tap water and was offered ad libitum. No substitution therapy was given to the adrenalectomized animals.

Blood pressure was determined by the plethysmographic technique. All animals had daily readings for a period of two to four days prior to surgery and at least once daily after surgery. The blood urea nitrogen level was determined prior to operation and again from a sample of heart blood at the time of killing.

An autopsy was performed on every rat, and microscopic sections were prepared from various organs. Because of the frequency of necrotizing vascular disease in the mesentery of the rat, this structure was generally embedded *in toto*.

### RESULTS

*Renal Infarction.*—Necrotizing vascular disease occurred in all 45 rats with renal infarction. However, the animals with greater reduction in renal substance, i. e., one kidney resected or totally infarcted, showed a somewhat wider distribution of vascular lesions than did those with one intact kidney.

As a rule, no vascular change was detected in the gross examination. Microscopically,

From the Institute of Pathology, Western Reserve University School of Medicine.

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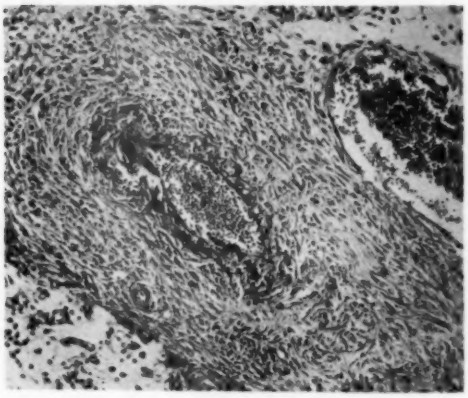


Fig. 1.—Polyarteritis nodosa in mesentery. Rat killed six days after ligation of anterior branch of left renal artery. Right kidney intact. Hematoxylin and eosin; reduced ⅓ from mag. × 124.

all animals showed necrosis of vessels, especially of the polyarteritis nodosa type, situated particularly in the mesentery and pancreas, occasionally in periadrenal tissue and heart, and infrequently in the stomach, large intestine, kidney, and testis. The commonest individual site was the mesentery enclosing the pancreas and attached to the stomach. The polyarteritis was found mainly in small arteries and arterioles and varied in distribution from sparse or focal to fairly extensive. It occurred in rats killed as early as two days after renal artery ligation. The necrosis involved most or all of the inner vessel wall, including muscularis and intima, and the cellular exudate usually extended through the entire wall.

In addition to polyarteritis, some animals showed fibrinoid necrosis of vessels, mainly arterioles and capillaries, with no proliferative or exudative component. Moreover, this constituted the principal lesion in a few rats killed at one or two days after surgery. Periarteritis without fibrinoid was also observed, but, since this lesion was found in several of the sham-operated rats, no special significance was attached to it.

About 40% of the animals showed myocardial necrosis, which was usually focal but

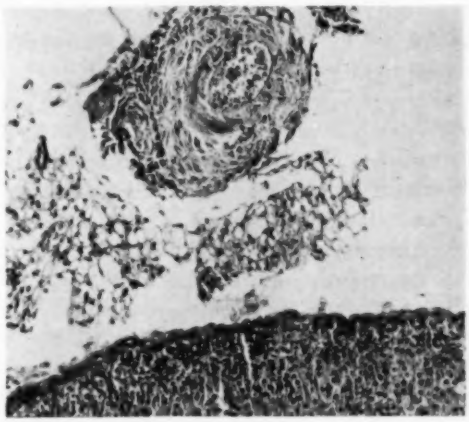
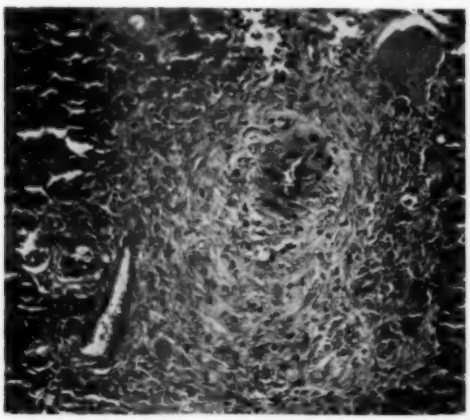


Fig. 3.—Polyarteritis nodosa in periadrenal tissue. Rat killed six days after ligation of anterior branch of left renal artery and resection of right kidney. Hematoxylin and eosin; stain reduced ⅓ from mag. × 156.

Fig. 2.—Polyarteritis nodosa in pancreas. Rat killed three days after ligation of anterior branch of left renal artery and resection of right kidney. Hematoxylin and eosin stain; reduced ⅓ from mag. × 184.



in a few instances involved almost the entire thickness of myocardium. The lesion was found in all portions of the right and left ventricular walls, especially the subendocardial region. The necrotic muscle fibers were generally homogeneous and deeply acidophilic, and their nuclei were shrunken, pyknotic, and distorted. Some lesions were associated with fibrosis, cellular exudate, or vascular necrosis. Similar necrosis was occasionally observed in the muscular layer of the gastrointestinal tract. No lesions were found in skeletal muscle or in the aorta.

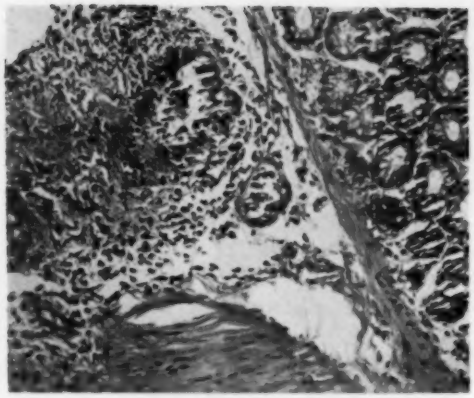


Fig. 4.—Polyarteritis nodosa in large intestine. Rat killed three days after ligation of anterior branch of left renal artery and resection of right kidney. Hematoxylin and eosin; reduced ⅙ from mag. × 184.

The majority of rats showed a distinct upward trend of the blood pressure following surgery. Many of those killed at five to seven days were already hypertensive, with blood pressures of from 175 to 190. However, in about 25% of the animals, mainly those killed prior to the fifth postoperative day, the blood pressure showed only a slight increment over the control readings, i. e., 20 to 30 mm. Hg. In addition, a small number of rats killed before the fifth day had no significant rise in blood pressure at all.

The blood urea nitrogen at the time of killing was usually normal or only slightly elevated. However, several animals with one kidney resected or totally infarcted had a moderately elevated level.

*Treatment with Antibiotics.*—The data on animals given antibiotics were similar in every way to those which did not receive this treatment. Since polyarteritis nodosa occurred in all these animals, it was evident that the antibiotic therapy did not influence the development of vascular lesions. This favors the view that infection is not a pathogenic factor.

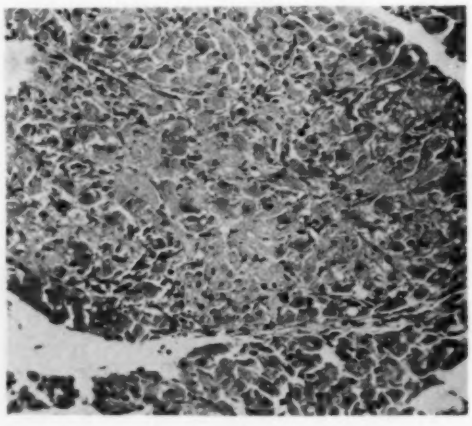
*Treatment with Dibenamine.*—The rats which received Dibenamine after renal artery ligation uniformly showed polyarteritis nodosa. The effect of the Dibenamine was to maintain blood pressure at normal levels throughout the postoperative experimental period, i. e., three to five days, in all but one rat. As with antibiotics Dibenamine did not interfere with the development of vascular necrosis.

*Sodium Space Studies.*—The six animals used to determine sodium space also showed necrotizing vascular disease. The values for this space, expressed as per cent of body weight, varied from 23.8 to 28.3, and the mean was 26.2. The corresponding values for normal rats, determined at the same time, were 25.5 to 27.3, with a mean of 26.4. Thus, there was no evidence that the presence of polyarteritis nodosa was associated with change in extracellular fluid volume.

*Effect of Total Adrenalectomy.*—The rats with simultaneous renal artery ligation and adrenalectomy did poorly after surgery. The majority lost weight progressively and were dead by one week. In most instances postoperative blood pressures were lower than the control values and were often in the hypotensive range, i. e., 80 to 100 mm. Hg. As a rule, the blood urea nitrogen at the time of death was elevated either moderately or markedly.

Fifteen of the 20 rats showed no evidence of necrotizing vascular disease, while 5 had polyarteritis nodosa. The vascular lesions were sparse and generally limited to the

Fig. 5.—Myocardial necrosis. Rat killed six days after ligation of anterior branch of left renal artery and resection of right kidney. Hematoxylin and eosin; reduced ⅙ from mag. × 215.



mesentery. The data indicate that adrenalectomy distinctly retarded the development of necrotizing vascular disease, although, unlike the effect on hypertension, it did not completely abolish it.

*Sham-Operated Animals.*—These showed no significant postoperative elevation of blood pressure. All blood urea nitrogen determinations were in the normal range. No instance of necrotizing vascular disease or myocardial necrosis was found. The mesentery usually revealed inflammatory exudate and fibrosis and, in several instances, focal granulomatous change and periarteritis.

## COMMENT

The principal type of vascular necrosis observed in this study was polyarteritis nodosa. Some animals also showed fibrinoid necrosis of vessels, with no exudative or proliferative component. The common development of these lesions after renal artery ligation suggests the possibility of a similar pathogenesis.

Polyarteritis is the vascular lesion usually observed in the rat after renal injury and hypertension, regardless of how the latter are induced. In the dog the principal change is fibrinoid necrosis rather than polyarteritis, even though an inflammatory and/or proliferative component may occur. The exudative and proliferative components in the rat, as well as the frequent mesenteric localization, probably represent peculiar species reactions.

Vascular necrosis has been observed in the dog * after total nephrectomy and bilateral ureteral ligation without special treatment designed to increase survival time. This has been confirmed for the rat in our laboratory. † About 50% of totally nephrectomized rats and 80% of those with bilateral ureteral ligation showed fibrinoid necrosis of vessels, mainly within mesentery and pancreas. However, as a rule, the lesions were sparse and were detected only after thorough search. For this reason simple renal ablation or ureteral ligation has limited value as techniques for the production of vascular necrosis.

The rapid production of extensive necrotizing vascular disease has been obtained in the dog by marked constriction of the renal arteries [3] or by total nephrectomy followed by use of the artificial kidney,[4] peritoneal lavage,[5] or intraperitoneal infusions of saline or Ringer's fluid.[6] So far, we have not found the last technique adaptable to the rat, since the life of the animal is not substantially prolonged and the vascular lesion is of the same sparse degree as that following nephrectomy alone. The other procedures are difficult or impractical in the rat. Kolff [7] used peritoneal lavage to prolong life in nephrectomized rats, but it is not clear that his results are significantly better than those from nephrectomy alone, from the standpoint of extent and frequency of the vascular necrosis.

Most procedures for inducing vascular necrosis in the rat without uremia require a period of time, i. e., weeks or even longer, for the uniform development of lesions. With constriction of one main renal artery [8] or silk perinephritis,[9] necrosis was sometimes observed within several days. Vascular disease also occurred rapidly after the injection of sulfonamides which produced renal obstruction.[10] The method of renal artery ligation has several advantages. Necrosis of vessels develops consistently within a few days, is usually prominent, and has no obligate association with uremia. Thus, a good means is provided, on a short-term basis, for study of the factors concerned with pathogenesis. Loomis [11] employed this method for the study of chronic hypertension in rats. Whether it is applicable to the dog is uncertain.[11]

Among the techniques which lead to vascular necrosis in the rat are constriction of a renal artery ‡; perinephritis §; figure-of-eight renal ligature ‖; renal artery ligation [11]; sub-

* References 1 and 2.

† Koletsky, S.: Unpublished data.

‡ References 8 and 12.

§ References 9 and 12 through 15.

‖ References 16 and 17.

total ablation of the kidneys ¶; temporary renal ischemia [20]; use of desoxycorticosterone,[21] anterior pituitary extract,[15] or vitamin D [22]; excessive salt intake [23]; choline deficiency [24]; alloxan poisoning [25]; administration of sulfonamides,[10] and parabiosis.# The majority of these procedures have in common a primary renal injury or reduction in renal mass, followed by hypertension and vascular disease. Damage to the kidney was accomplished in such ways as reduced blood flow, dietary means, or toxic agents, although in some instances the mechanism of injury is not clear. An exception is Zeckwer's report that vascular necrosis occurred in 3 out of 20 pairs of parabiotic rats with essentially intact kidneys.[27]

While experimental hypertension in the rat is probably initiated in the kidney, the origin of the vascular lesions commonly associated with elevation of the blood pressure is still obscure. As with hypertension, the problem of pathogenesis is complicated by the fact that vascular necrosis can be produced either in the presence or in the absence of the kidneys. Whether the origin is similar or different for each situation is unknown. None of the theories of origin proposed so far are applicable under both conditions. No common renal dysfunction bearing on pathogenesis has been established.

Experimentally, the close association of hypertension and necrotizing vascular disease has often led to the conclusion that the elevated blood pressure caused the vascular change. The evidence here is essentially indirect, since it rests on exclusion of other possible etiologic factors such as hypersensitivity, infection, and uremia. Very little has been advanced as to how elevated blood pressure induces necrosis of vessel walls except the proposal by Byrom and Dodson [28] that increased tension acts through overdistention of the vascular wall. This work is not convincing and, moreover, was not confirmed in either the rat [29] or the dog.[30]

¶ References 18 and 19.

# References 26 and 27.

It is generally accepted that no obligate connection exists between hypertension and necrotizing vascular disease. In the rat the hypertensive state may exist for a substantial time without any vascular disease, and, conversely, vascular necrosis has been reported in animals which were not hypertensive. The belief that development of the vascular lesion requires a rapid increment in blood pressure is without sound foundation, and the same appears likely for the requirement of a high level of pressure, or one which is sustained for some time.* The suggestion that hypertension, while not responsible for the vascular disease, serves as an augmenting factor may be valid, but whether this occurs by a direct effect or by indirect means is not known.

In this study several observations indicate that hypertension and vascular necrosis can be dissociated and that hypertension is not an essential causative factor: 1. Vascular lesions were observed in several animals as early as one or two days after operation, when no rise in blood pressure had occurred. 2. Although many rats which showed polyarteritis nodosa three to five days after renal artery ligation had an elevation of blood pressure, the increment over the control readings was small, i. e., about 15 to 20 mm. Hg. 3. Vascular lesions occurred in rats whose blood pressure was maintained at the control levels by daily administration of Dibenamine. 4. Polyarteritis nodosa was occasionally observed in totally adrenalectomized animals, which were distinctly hypotensive throughout the experimental period.

In addition, the fibrinoid necrosis in rats with total nephrectomy or ureteral ligation cannot be attributed to hypertension, since the latter was absent in all these animals.†

There is ample evidence in the rat that vascular necrosis does not depend on loss of renal excretory function. Loomis [11] demonstrated this in convincing manner in rats with renal infarction, and her experience was confirmed in the present study. Florid vascular necrosis follows other procedures,

* References 7, 13, 14, and 31.

† Koletsky, S.: Unpublished data.

such as administration of desoxycorticosterone, without even incipient uremia. Moreover, necrotizing vascular disease can be produced rapidly, as in this study, with only a 25% reduction in total renal mass, i. e., necrosis of one-half of one kidney. However, this does not obviate the fact that with a greater reduction in renal substance the vascular lesions were more pronounced and widespread.

The absence of a significant association in the rat between necrotizing vascular disease and renal excretory failure is in contrast to the situation in man. Widespread arteriolar necrosis, together with rapidly progressive uremia, is characteristic of malignant human hypertension. The necrosis is superimposed on chronic vascular lesions, i. e., those of benign hypertension, such as intimal fibrosis, elastic reduplication, and medial hypertrophy.[32] That the latter, especially arteriolar sclerosis, have a definite counterpart in the rat is not clearly established. Vascular lesions associated with hypertensive renal disease in the rat are essentially necrotizing from the start, even when there is no question of adequate renal excretion, and give rise by fibrosis and hyalinization to the chronic or healed types of vascular disease. These are generally distinct from true intimal arteriosclerosis as such.

In the dog, also, necrotizing vascular disease is more closely associated with uremia, even granting that the latter is not a primary requisite. Maintenance of the blood urea nitrogen of nephrectomized dogs at moderately elevated levels by peritoneal lavage would appear not to alter the basic condition of renal excretory failure.[30] The rat differs in that vascular necrosis occurs both in benign hypertension and in what might be termed malignant hypertension.

The view that necrosis of vessels might result from the direct action of a necrotizing substance derived from kidney is attractive but hypothetical. Loomis [11] stated that both vascular disease and hypertension in her animals with renal infarction arose in this way, but she offered no supporting evidence. Winternitz and associates [1] observed vascular necrosis in nephrectomized dogs injected with saline extracts of necrotic dog kidneys. The necrosis was more extensive than after nephrectomy alone. However, there were similar lesions with extracts of normal kidneys and also of testis. Such results are inconclusive, but the role of a necrotizing agent deserves further study. Of course such an agent could not explain the vascular necrosis which follows total nephrectomy.

The present study provides some preliminary information on the role of the adrenals in the production of necrotizing vascular disease. Rats subjected to simultaneous renal arterial ligation and adrenalectomy survived only a short time, about one week, if no substitution therapy in the form of saline or corticosteroids was given. Under the latter condition adrenalectomy markedly diminished the incidence of polyarteritis nodosa but did not abolish the lesions entirely. However, it uniformly abolished the development of hypertension. This indicates that the inhibition of vascular necrosis by adrenalectomy was not necessarily achieved through lowering of the blood pressure. Such inhibition may have been related to the very poor general condition of the animals after adrenalectomy. Perhaps the alterations in electrolyte and water metabolism which follow adrenalectomy played a role, as has been suggested for the dog.[33]

The chemical changes associated with experimental hypertension and renal disease have been investigated. Eichelberger [34] demonstrated an increased sodium and chloride content, a decreased potassium content, and extracellular edema in the muscle of hypertensive dogs. Hypertensive rats showed similar electrolyte changes in muscle, a decrease in chlorides in other tissues, little change in total water content of tissues in early hypertension, and greatly increased content in the later stage.[35]

Several authors have linked electrolyte shifts and/or change in extracellular fluid space with the development of vascular necrosis and hypertension. For example,

Turner and Grollman [35] postulated loss of a mechanism controlling sodium and potassium ratio between cells and interstitial fluid. Some parallel may exist between the myocardial necrosis of rats with experimental potassium deficiency ‡ and that of animals with hypertensive renal disease. The muscular location of the lesion is perhaps significant, especially since the fibrinoid necrosis of vessel walls also involves the muscular layer. The implication is that release of cellular potassium might be a factor in the pathogenesis of such lesions. However, there is no direct evidence that such is the case. The myocardial necrosis in acute potassium deficiency may be due to concomitant renal damage.[37] Further clarification is needed as to the manner in which electrolyte shifts insert into the chain of events between renal injury (or ablation) and the development of vascular necrosis.

Other workers have emphasized the pathogenic role of increase in the extracellular fluid compartment. In studying nephrectomized dogs kept alive by peritoneal lavage, Leonards and Heisler [39] concluded that the development of hypertension was dependent on retention of fluid and expansion in extracellular fluid volume. Increase in this space, together with altered electrolyte content, was also emphasized by Orbison and co-workers,[6] who studied nephrectomized dogs given large amounts of intraperitoneal fluid. However, Grollman [40] attributed the rise in blood pressure obtained by the last two groups of authors to correction of extracellular fluid deficit induced by the conditions of their experiment rather than to expansion of the extracellular fluid volume. He also found that marked increase of the extracellular fluid space had a relatively insignificant effect on the blood pressure of dogs made hypertensive by the figure-of-eight renal ligature.

Our studies so far indicate that significant expansion of the extracellular fluid space is not a necessary accompaniment of vascular necrosis. It would appear unlikely that increase in this space serves as a generally operative mechanism for the induction of necrotizing vascular disease or hypertension, especially in those techniques which do not involve total nephrectomy and administration of fluid.

As this investigation shows, vascular necrosis in the rat follows within a few days after renal infarction and can occur without hypertension, without uremia, and in the absence of adrenals. Essentially what remains as the probable primary source of the vascular lesions is the renal injury. The latter possibly yields a necrotizing agent or produces a renal dysfunction which leads to vascular necrosis. The dysfunction of the kidney may be related to altered electrolyte metabolism.

‡ References 36 through 38.



## SUMMARY

Infarction of the rat kidney was followed within a few days by the development of necrotizing vascular disease.

With this technique it was readily shown that the necrosis of vessels was not dependent on hypertension or renal excretory failure. The lesions occurred without expansion of the extracellular fluid space. They were not prevented by antibiotics. The vascular disease was diminished in frequency but not abolished by total adrenalectomy.

The injury to the kidney was evidently the primary pathogenic factor in the production of the vascular necrosis. The possibility that a necrotizing substance derived from the kidney was responsible is neither established nor excluded. Otherwise, some form of renal dysfunction, resulting from the injury, probably played a role, and, in this connection, disturbances in electrolyte metabolism may be significant.

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# News and Comment

## ANNOUNCEMENTS

**Institute of Dental Medicine Annual Meeting.**—The next Annual Meeting of the American Institute of Dental Medicine will take place at the Desert Inn, Palm Springs, Calif., Oct. 23 to 27, 1955. The faculty will consist of:

Maury Massler, D.D.S., M.S., University of Illinois, Chicago: Effects of Filling Materials on the Pulp; Sterilization of Dentin; Tissue Changes During Aging.

Valy Menkin, M.D., Temple University School of Medicine, Philadelphia: Inflammation, Bacterial Invasiveness and Immunity; Chemical Factors Liberated by Injured Cells in Inflammatory Exudates; The Anti-Inflammatory Problem with Particular Emphasis on the Mechanism Concerned, as Induced by Corticosteroids.

Hans Selye, M.D., University of Montreal, Montreal, Canada: Recent Progress in the Study of Stress as Applied to Dental Medicine.

Reidar F. Sognnaes, D.M.D., Ph.D., Harvard School of Dental Medicine, Boston: Relative Significance of Cellular and Chemical Remodeling of Bones and Teeth as Revealed by Radioactive Isotopes; Complexity of the Caries Problem Suggested by Recent Observations in Experimental Animals; Clinical Implications of Experimental Caries Research.

Wendell L. Wylie, D.D.S., University of California, San Francisco: Tooth Guidance and Factors Influencing the Rate of Eruption.

All Seminar lecturers will participate in a round table forum discussing the application of their subject to the practice of Dental Medicine. Applications and full information may be secured from the Executive Secretary, Miss Marion G. Lewis, 2240 Channing Way, Berkeley 4, Calif.

# Biophysical Studies of the Bone Tissue of Dogs with Experimental Rickets

B. ENGFELDT, M.D.
and
R. ZETTERSTRÖM, M.D., Stockholm

IN RICKETS there is a failure in calcification while the growth of the bones continues, although at a slower rate. In this way osteoid, i. e., uncalcified, tissue is formed both in the metaphyseal zone and in the bone formed prior to the deficiency, in which remodeling processes are going on. Despite numerous extensive studies with chemical and histological methods (for relevant references cf. Weinmann and Sicher [1]), the cause of the inability of the hypertrophic cartilage in rickets to calcify is not quite elucidated, and there are many divergent opinions. The main question is whether the disturbed mineralization is due only to a decreased Ca×P quotient in the serum, and consequently also in extracellular fluid, or whether there is in addition some primary disturbance in the bone-forming tissue.

In earlier investigations the distribution of bone minerals has been studied in undecalcified sections after silver impregnation (von Kóssa [2]; McLean and Bloom [3]) or by staining with alizarin (Cameron [4]). In decalcified sections the staining of the organic matrix has given information concerning the mineral distribution. Although Urist and McLean have found that the distribution of bone salt, as demonstrated by the silver method, is identical with that found by intravitam staining with sodium alizarin sulfonate (alizarin red S), the impregnation with silver is not specific for calcium phosphate (Cameron [4]). It is also impossible with the methods used earlier to secure more quantitative information concerning the amount of mineral salts deposited in different structures of the bone tissue.

In the present paper the results of studies of the bone tissue in experimental rickets by means of various biophysical methods will be reported. The distribution and relative content of mineral salts in different structures of the bone have been studied with the aid of microradiography. The uptake of labeled phosphate and calcium has been studied in vivo and in vitro by use of radioautography. The nature of the mineral salts, their crystalline structure, and their orientation have been investigated with wide-angle x-ray diffraction, and the arrangement of the collagen in decalcified sections, with polarized light. By applying these methods along with ordinary histological ones, it has been possible to obtain more accurate information concerning in what manner the calcification in rickets differs from that in normal conditions. In this investigation only mild rickets has been produced, in order that secondary changes, difficult to interpret, might be avoided.

## MATERIAL

Three dogs of the same litter, 2½ months old, were used in these experiments. Two of them were put on a rachitogenic diet according to Mellanby [5] and were placed in a cage in complete darkness. The third dog served as a control. After one month on the rachitogenic diet, when the dogs were 3½ months old, they had developed mild rachitic changes, as disclosed by x-ray examination. The dogs were then given intravenous injections of 1 mc. of labeled phosphate per kilogram of body weight,

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From the Department of Physical Cell Research and the Department of Pathoanatomy, Karolinska Institutet, and the Pediatric Clinic, Karolinska Sjukhuset.

viz., $Na_2HP^{32}O_4$ containing negligible amounts of nonlabeled phosphate, and were killed after three hours.

## METHODS

The histological investigation of the osseous tissue was performed both with specimens which were immediately decalcified, embedded, sectioned, and stained according to routine methods, and with ground sections. Thin longitudinal sections from the epiphysis and the metaphysis of the long bones and cross sections from the diaphysis were cut out with the aid of a rotating saw. These sections were ground on glass plates to a thickness of about 50 to 100 $\mu$.

The content and distribution of mineral salts in ground sections of the bone tissue were determined by microradiography with, in principle, the procedure described by Engström and Wegstedt.[6] The radiation source for small samples was a Machlett AEG 50 x-ray tube operated at 6 to 8 kv. The radiation was filtered in 1 mm. Be. For larger samples a Machlett OEG 50 x-ray tube with a 0.2 mm. Be window was used. This tube was run with a 24-kv., fully rectified direct current. With the radiation thus obtained, more than 97% of the absorption in the ground sections was due to calcium salts. The x-ray image was recorded on an extremely fine-grained emulsion (Lippman film or Eastman Kodak Spectroscopic Plate No. 649) with a resolving power of less than 1 $\mu$. The microradiograms were enlarged by microphotography on Kodak O 250 plates.

After microradiography the sections were used for radioautography in order to study the localization of the in vivo uptake of labeled phosphate. When, owing to decay, the radioactivity had completely disappeared from the bone tissue, the exchangeability of the calcium ions in the bone minerals was investigated in vitro. The ground bone sections were incubated in $Ca^{45}Cl_2$ solution according to a method earlier described (Engfeldt[7]). The radioautograms were obtained by sandwiching the ground sections between sheets of Agfa Printon film. A special filter holder was used in order to secure good film-specimen contact. The films were exposed for about two days and were then developed in Kodak D 19 b. After having been dried, the films were mounted on slides in Canada balsam and covered with a coverslip. The radioautograms were enlarged by photomicrography on Kodak B 20 plates.

The ultrastructure of the bone tissue was investigated with x-ray diffraction, using a Hilger 190 mm. cylinder camera adapted to a Siemens Crystalloflex II diffraction unit. Nickel-filtered Cu K-radiation was used. For the investigation of the ultrastructure of microscopical areas, a Chesley[8] microcamera was used. This permitted the investigation of areas as small as 50 $\mu$ in diameter.

For microphotography in transmitted and in polarized light (crossed Nicol prisms) the sections were then decalcified in formic acid and mounted in 5% sodium sulfate. Afterward they were stained for histological investigation.

## RESULTS

*Changes in the Growth Zone.*—The histological examination of the growth zone in the rachitic puppies revealed a broadened and somewhat irregular epiphyseal plate. As is shown in Figure 1, the vessels are dilated in the metaphyseal areas. The invading mass of blood vessels seems to have destroyed both cartilage cells and part of the matrix substance. In consequence of these changes, there is between the epiphyseal plate and the bone tissue a zone containing mostly dilated vessels, which irregularly penetrates the cartilage. The findings are the same as those earlier described in detail by Dodds and Cameron[9] and by Park.[10] The bone trabeculae in the metaphyseal areas are irregularly arranged and covered by ample osteoid tissue, which is outlined by an abundance of osteoblasts.

In Figure 2, *A* and *B* are low-power views of the microradiograms from the growth zone in a metatarsal bone of a normal and a rachitic puppy, respectively. In the epiphyseal plate there is no deposition of bone minerals, this area thus being transparent to x-rays. It is plainly apparent that this zone is much broader in the rachitic puppy than in the control. The calcified trabeculae are more regularly arranged in the normal puppy than in the rachitic one.

In Figure 2, *C* and *D* are high-power views of the sections shown in Figure 2 *A* and *B*. In the normal puppy, there is a regular arrangement of the calcified tissue. When the picture is compared with the decalcified and stained section (Fig. 1 *A*), it will be seen that the bone salt is deposited chiefly corresponding to the longitudinal part of the intercellular matrix which separates the rows of cartilage cells. In addition, in the zone of calcification there is deposition of calcium

salts in the cross partitions between the cells. These findings are in agreement with results reported by Dodds [11] and by others. The microradiographic patterns also demonstrated that the calcification keeps a few cells ahead of the invading capillary loops and connective tissue; this observation has earlier been made by Dodds and Cameron,[9] using other techniques. The microradiographic investigation shows that the content of mineral salts is very low in the zone of calcification, but from the microradiographic patterns to deduce the site of the vanished chondrocytes. In places where the cartilage tissue has persisted, the pattern of the calcification is more irregular.

In rickets (cf. Fig. 2 *D*) the distribution of the bone minerals seems to lack normal organization. In the primitive spongious bone the trabeculae are unevenly calcified; in some parts there is high mineralization, and in others the content of mineral salts is very

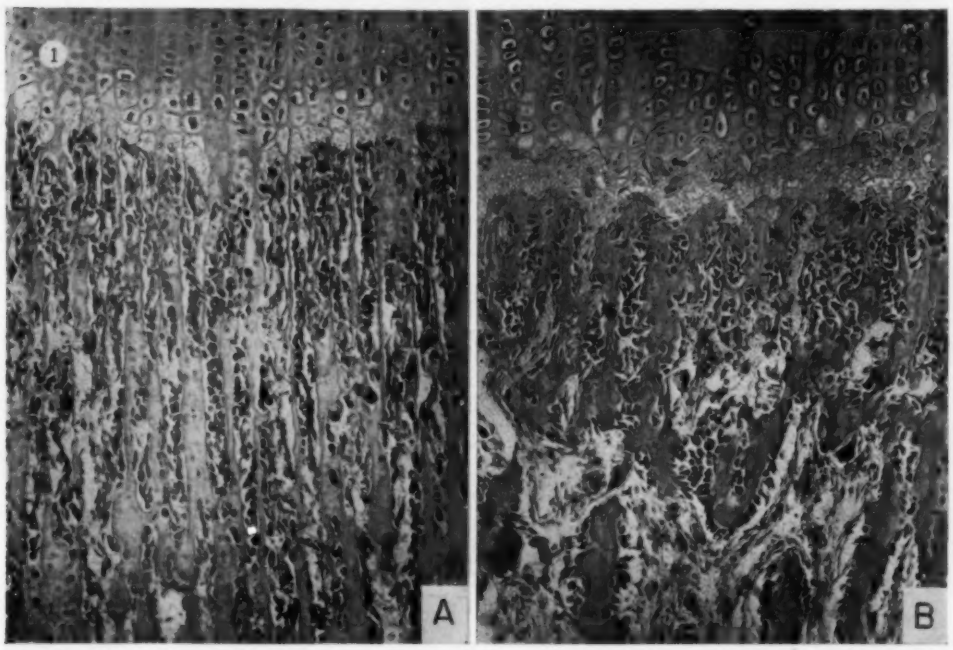



Fig. 1.—Longitudinal decalcified section through the epiphyseal plate and the metaphyses of the metatarsal bone from (*A*) normal dog and (*B*) dog with experimental rickets. Note the wide capillaries invading the cartilage and the irregular, clumsy bone trabeculae in the metaphyses. Van Gieson stain; reduced ⅓ from mag. × 200.

that it increases at a comparatively rapid rate. However, in each calcified column the calcification intensity is very uneven; in some parts there is a high degree of calcification, and in other parts the mineralization is low. Usually the calcification is poorly delimitated from the marrow spaces. The regularity of the calcified trabeculae depends upon the dissolution of the chondrocytes and the transverse calcified intercellular bars. Even if this resorption is almost complete, it is possible low. In some of the trabeculae there are completely uncalcified areas, which correspond to remnants of cartilage cells. The microradiograms also show that there are poorly calcified areas in the fibrous marrow tissue. The irregularity in the calcification and the arrangement of the calcified matrix might be due to the disappearance of the degenerative cartilage and, above all, the longitudinal intercellular bars adjacent to the metaphysis. In rickets the formation of bone

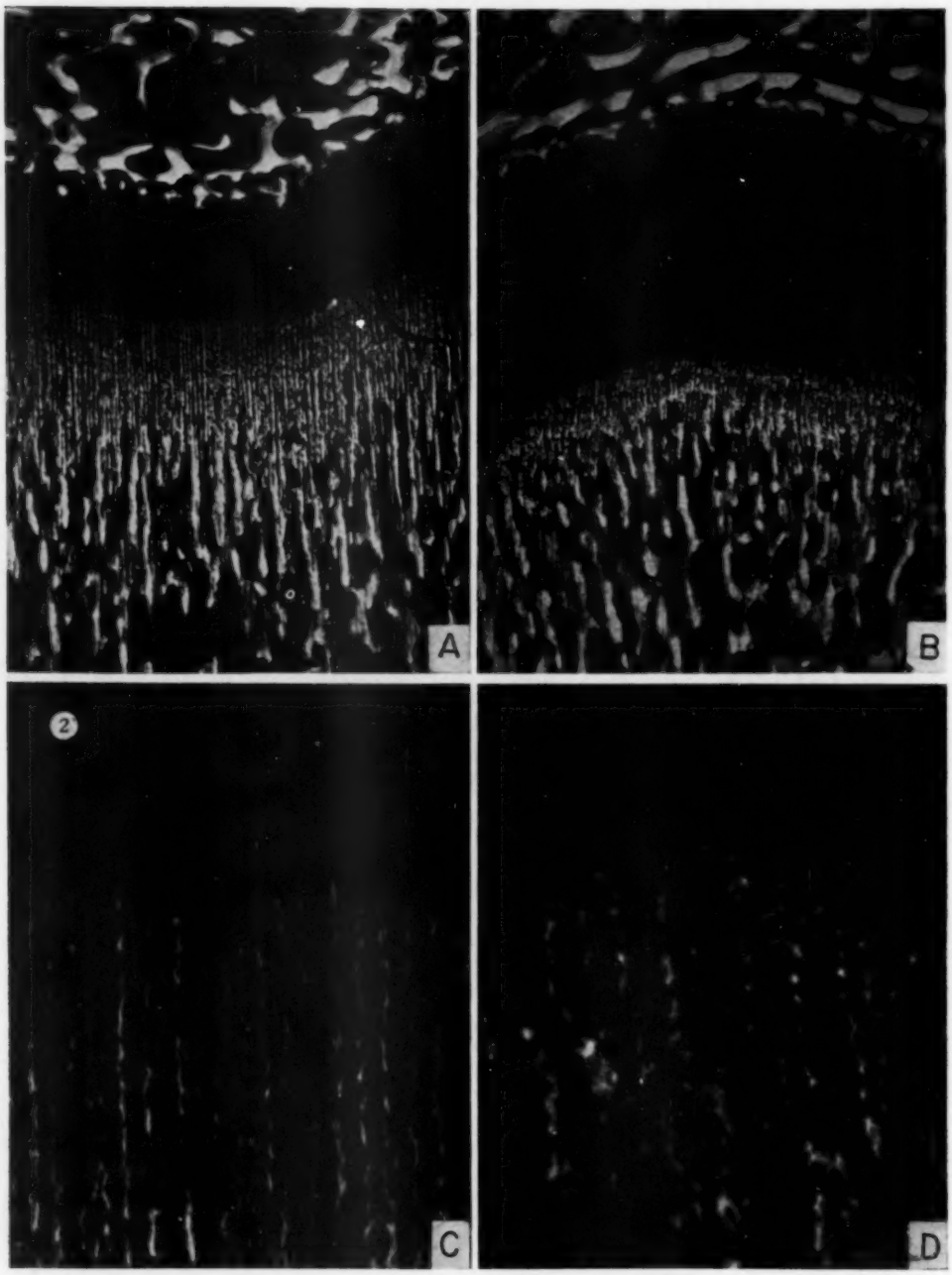



Fig. 2.—Microradiograms of ground thick longitudinal sections (100 μ thick) from the epiphysis and metaphysis of metatarsal bone from normal (*A* and *C*) and rachitic (*B* and *D*) dogs. In *A* and *B* the difference in width of the epiphyseal plate between the control and the rachitic dog is evident. Reduced ⅓ from mag. × 20. *C* shows the highly ordered bone trabeculae in the metaphyses of a normal dog. In *D* the irregularity of the bone trabeculae in the rachitic metaphysis is demonstrated. Reduced ⅓ from mag. × 200.

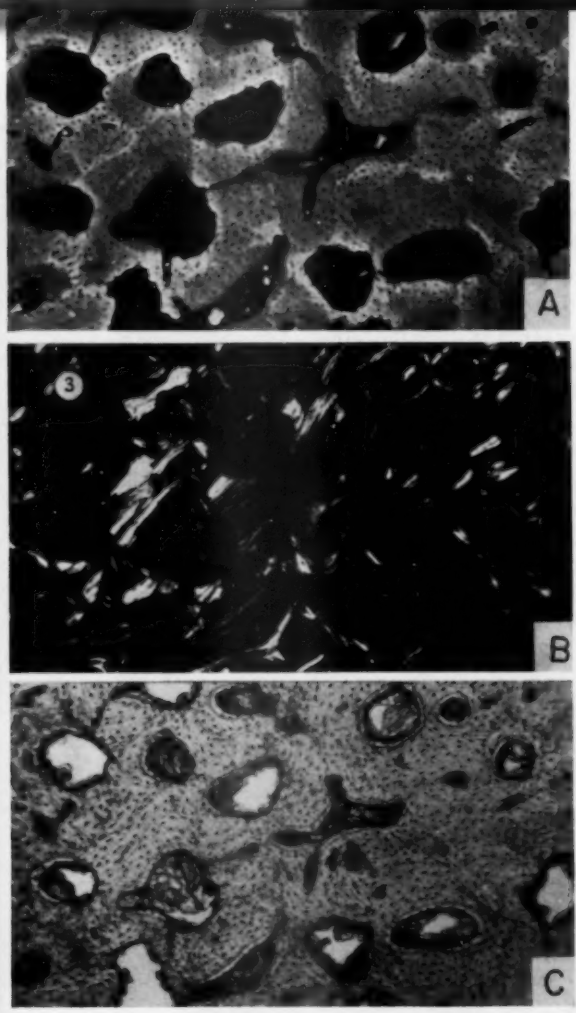
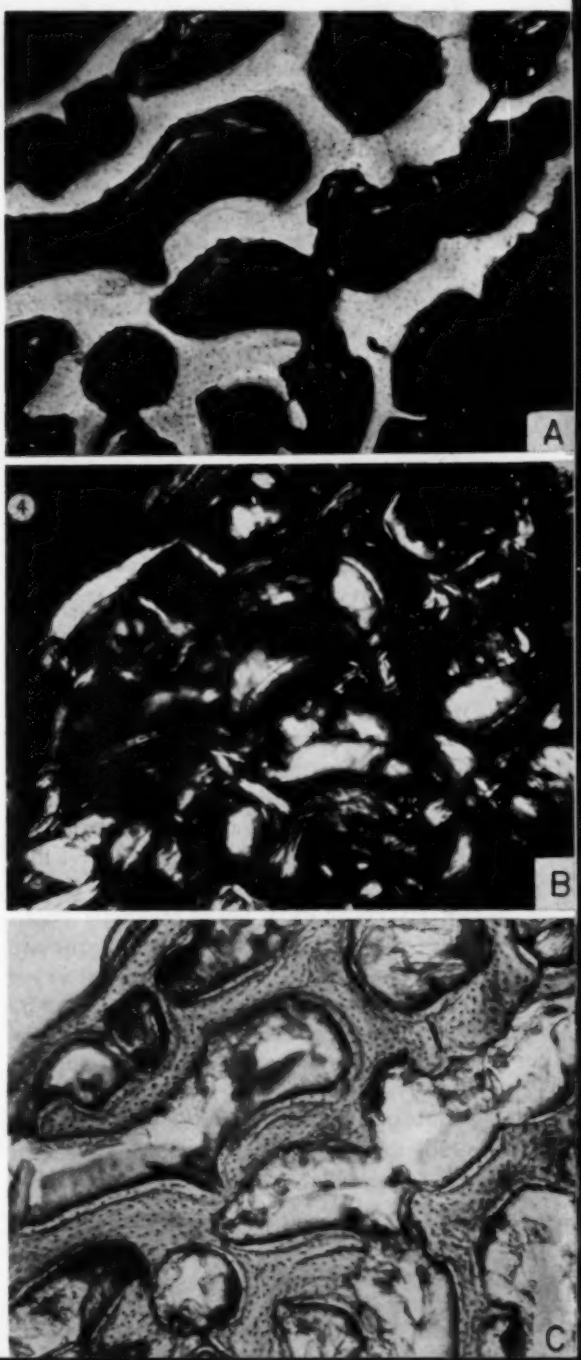



Fig. 3.—Transverse ground section (50 μ) from the diaphysis of the tibia of the control dog; reduced ½ from mag. × 120. Microradiogram (*A*) shows the distribution of mineral salts. The osteocytes appear as dark spots. *B* and *C* show the same section decalcified and photographed in polarized light (*B*) (crossed Nicols prisms) and in transmitted light (*C*). The well-organized collagen pattern is demonstrated in *B*.

trabeculae thus goes on without the check which is normally provided by the regular arrangement of the cells in the metaphyseal plate.

In the growth zone, there is in rickets a continued growth with increasing thickness of the epiphyseal plate. The vascular invasion into the cartilage is delayed and abnormal. When, however, the cartilage has been penetrated by vessels, irregularly calcified and abnormally arranged trabeculae including remnants of chondrocytes are formed.

Fig. 4.—Transverse ground section (50 μ) from the diaphysis of the tibia of a rachitic dog; reduced to ⅓ from mag. × 140. In the microradiogram (*A*) the thin mineralized line in the resorption cavities separated from the highly mineralized bone trabeculae by a nonabsorbing zone is well demonstrated. *B* and *C* show the same section decalcified and photographed in polarized light (*B*) and in transmitted light (*C*). The broad osteoid zone shows a fiber pattern which is different from that of the adjacent bone trabeculae.



*Changes in the Compact Bone.*—Histological examination of transverse sections from the diaphysis of the long bones taken from the rachitic puppies disclosed large amounts of osteoid tissue in the periosteal and endosteal areas. The compact bone tissue showed an abundance of wide, irregular resorption cavities, and many Haversian systems were lined with osteoid tissue toward the canal.

Figure 3 shows the microradiogram (*A*), a photomicrograph in polarized light (*B*), and a photomicrograph in transmitted light (*C*) at the same magnification of the same cross section of the tibial diaphysis of the control puppy. Owing to the high rebuilding intensity, many large cavities are found in this normal bone. As has been shown with microradiographic technique in an earlier investigation (Amprino and Engström [12]), the compact bone is not uniformly calcified; i. e., the absorption of x-rays varies in different parts of the calcified bone structure. Different Haversian systems display varying degrees of mineralization, the content of mineral salts being highest in the oldest structures of the bone. The collagen in the compact bone of this normal puppy is regularly arranged, as disclosed by the photomicrograph in polarized light (Fig. 3 *B*). The photomicrograph in transmitted light (Fig. 3 *C*) shows that most of the cavities are entirely or partly lined with a thin zone, well defined against the outer parts of the bone tissue and containing osteocytes. When the picture in transmitted light is compared with the microradiogram and the diameters of the cavities are measured, it can be demonstrated that this zone (10 to 20 $\mu$ in breadth) shows a thin line of mineral salts toward the Haversian canal but is otherwise uncalcified. The collagen fibers in this area are regularly arranged in the same way as in the rest of the osteon. This zone can be correlated to the lamellar arrangement of the organic and inorganic constituents of the Haversian system, as demonstrated by Engström and Engfeldt.[13]

Figure 4 *A* is a microradiogram from a ground cross section of the tibia from a rachitic puppy. As in the compact bone tissue from the normal puppy, there are areas of varying mineralization. Resorption cavities with uneven borders are widely formed, and these cavities cover bone tissue of different degrees of mineralization. The osteomalacic changes are more or less pronounced in different parts of the skeleton. As is shown in Figure 4*B*, there are in some parts of the bone structure well-organized collagen fibers constituting typical Haversian systems in the old bone formed before rickets was induced. The so-called osteoid tissue in the cavities has

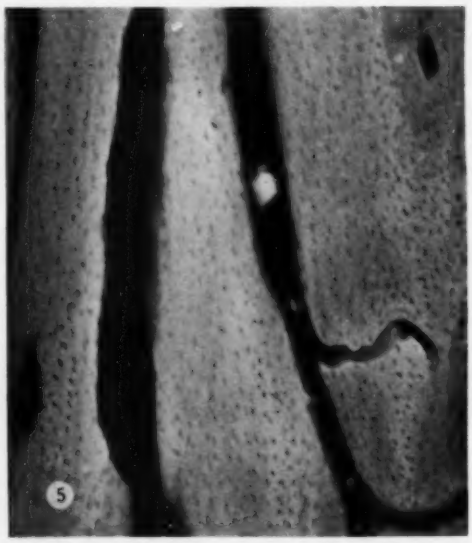



Fig. 5.—Microradiogram of a longitudinal ground section (70 $\mu$) through the compact bone of the tibial diaphysis from a rachitic dog showing cavities with osteoid tissue; reduced ⅓ from mag. × 100.

a varying collagen content, the variations being great also in different parts of the same osteoid border. In some parts the collagen content is high, whereas in others it is very low. Instead of concentric layers of collagen fibers, there are short, broad bundles often running perpendicular to the canals.

A comparison of the microradiogram and the photomicrographs in polarized and transmitted light (Fig. 4 *A*, *B*, and *C*) shows that in rickets, as well as under normal conditions, there is a thin, mainly uncalcified, zone with a well-organized collagen pattern outlining

the cavities. This zone is well defined not only against the calcified tissue but also against the abundant osteoid tissue filling great parts of the cavities. The so-called osteoid tissue either is completely uncalcified or has a very low mineralization. A very common feature of this area is, however, a very thin but definitely mineralized line constituting the border toward the soft tissue.

Figure 5 is a longitudinal section of compact bone from one of the rachitic puppies. In this Figure it is shown, as is also demonstrated in Figure 4A, that there is a mineralization in the osteoid tissue, though it is very low except in thin areas, perhaps corresponding to the outer lamellae.

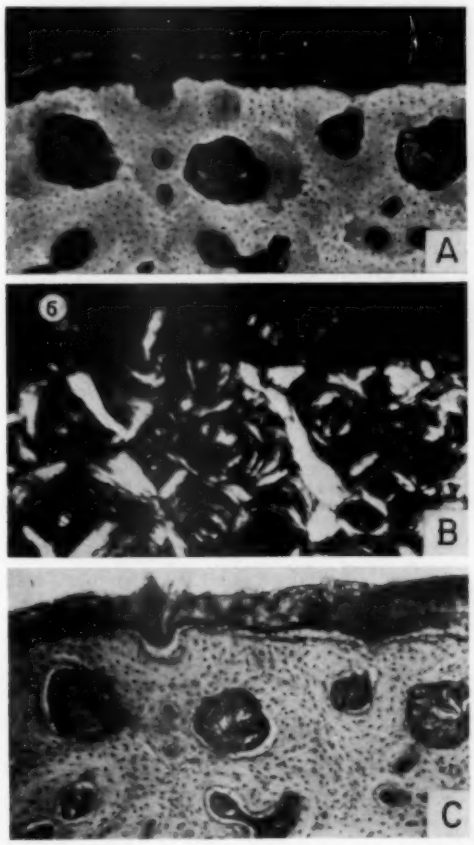



Fig. 6.—Ground transverse section (80 $\mu$) from the periosteal area of the diaphysis of the metatarsal bone from a rachitic dog. In the microradiogram (*A*) note the thin mineralized line separated from the highly mineralized bone tissue by a nonabsorbing zone in the periosteal area. *B* and *C* show the same section after decalcification photographed in polarized light (*B*) and in transmitted light (*C*). Reduced ⅓ from mag. × 120.

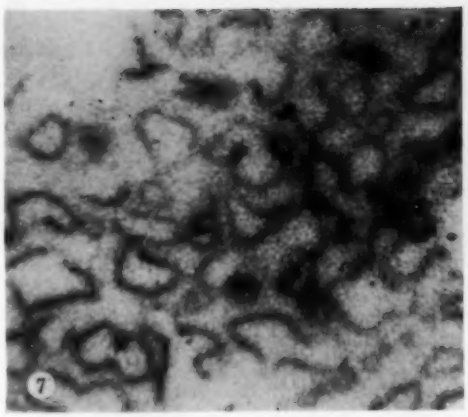



Fig. 7.—Radioautogram of a transverse ground section of compact bone from a rachitic dog labeled in vivo with phosphate. A high uptake of isotope in the tissue lining the cavities is evident. Reduced ⅓ from mag. × 35.

Figure 6 shows the microradiogram (*A*) and the photomicrographs, in polarized (*B*) and in transmitted light (*C*), of a cross section from the periosteal portion of a diaphyseal bone from a rachitic puppy. In this section the bone structure is more normal than in the section shown in Figure 4. According to the image in polarized light, the collagen pattern shows no abnormalities. Also, in this section there is toward the cavities a distinct lamellar zone which, according to the image in polarized light, has a normal organization of the collagen bundles. From the microradiogram it will be seen that this area has an extremely low mineralization, or completely lacks calcium salts. In the subperiosteal zone, there is abundant osteoid tissue with a low content of poorly organized collagen. Apart from a thin, rather highly mineralized zone in the outer portion of this border of osteoid tissue, the mineralization is very low or absent in this area. The findings in the periosteal layers are thus principally the same as those found in the cavities of the compact bone.

Figure 7 is a radioautograph of a cross section of compact bone from a rachitic puppy, demonstrating the uptake of labeled

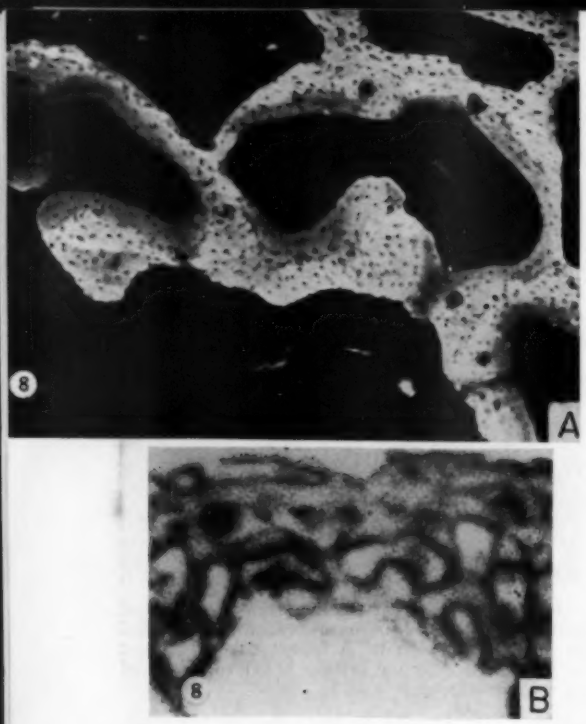



Fig. 8.—Transverse ground section from compact bone of a rachitic dog. The section was incubated in vitro with $Ca^{45}Cl_2$. Microradiogram (*A*), reduced ⅓ from mag. × 140, shows a detail of the section, the radioautogram of which is shown in *B*, reduced ⅓ from mag. × 40. Note the uptake of isotope in certain structures, viz., those with a low degree of mineralization. The thin mineralized line in the endosteal area also shows a detectable uptake of $Ca^{45}$ (in the lower part of the specimen).

phosphate in vivo three hours after injection of the tracer phosphate. It is clearly demonstrated that the radioactivity is deposited in the calcified tissue surrounding the cavities and that the labeling differs around various cavities. In many the uptake is high in some parts of the circumference but extremely low in others. The uptake is localized at a rather distinct and narrow zone toward the cavities. The uptake of radioactivity is also high in the periosteal areas. The bone tissue between the cavities shows minimum labeling. In an earlier investigation (Engfeldt, Engström, and Zetterström [14]) it has been shown that the uptake of labeled phosphate in the compact bone is highest in young Haversian systems, with a low content of mineral salts. When the systems grow older and the amount of mineral salts approaches a maximum value, the uptake of radioactive phosphate becomes very low. In the resorption cavities the uptake is negligible. The high, rapid uptake of the labeled phosphate in certain structures of the bone tissue was thought to be due mainly to ionic exchange. Since in rickets the calcification process is disturbed, there is low mineralization of newly formed bone tissue. Consequently, there is a retardation of the increment in calcification that under normal conditions is found in the growing Haversian systems (Amprino and Engström [12]). The cessation of this normal process leads to a persistence of the high exchangeability of ions constituting the bone minerals. Owing to this fact, the uptake of injected labeled phosphate in short-time experiments is of the same order of magnitude in rickets as under normal conditions, although in rickets new deposition of bone minerals is low. Claassen and Wöstmann [15] have earlier arrived at the conclusion that the first rapid uptake by exchange is not changed appreciably in rickets. Owing to the reduced new deposition, however, the labeling becomes lower in rickets than under normal conditions in long-term experiments, as has also been pointed out earlier (Copp and associates [16]).

A microradiogram and a radioautograph obtained with the in vitro technique of the same cross section from the compact bone of a rachitic puppy is shown in Figure 8. Areas with low content of mineral salts show a high uptake of radiocalcium, while resorption cavities are not being appreciably labeled. Corresponding to the broad osteoid border at the endosteal surface, a thin mineralized zone can be detected at its inner border, and the radioautogram reveals a definite uptake corresponding to these areas.

X-ray diffraction studies of the bone tissue from the rachitic dogs have shown that it contained mineral salts given a pattern characteristic of hydroxyapatite. The unit cell revealed the same dimensions as is found in normal bone, i. e., a-axis 9.41 Å. and c-axis 6.87 Å. The c-axis is oriented along the fiber axis of the collagen. Osteoid borders from the transverse sections have been separated from the adjacent tissue and submitted to micro x-ray diffraction. The diagrams thus obtained showed an apatite pattern.

The diffraction lines were diffuse, indicating a small particle size, a result always found in osseous tissues. After heating to 600 C. for one hour, the pattern became sharp, owing to the growth of the crystallites. The diffraction pattern showed the unexpected result, that the hydroxyapatite crystallites have a preferential radial orientation. A micro x-ray diffraction diagram obtained from a cross section of the osteoid tissue is shown in Figure 9.

## COMMENT

From our results it is evident that, although being arrested in rickets, mineralization of the newly formed organic matrix of the bone tissue is not completely lacking. We have thus been able to demonstrate in the microradiograms a thin zone of higher x-ray absorption at the outer border of the osteoid tissue. This higher x-ray absorption indicates a deposition of mineral salts. Micro x-ray diffraction of this area has also shown the presence of apatite, and in our in vitro ionic exchange experiments we were able to demonstrate an uptake of $Ca^{45}$ in this zone.

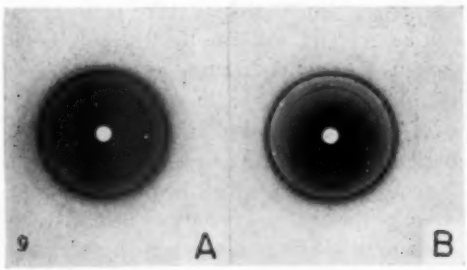



Fig. 9.—Micro x-ray diffraction diagrams obtained from the osteoid tissue showing definite fibering. *A* shows tissue before, and *B* after, heating.

In the metaphyseal growth zone certain amounts of mineral salts are also deposited, as is shown by the microradiograms, though this occurs irregularly, and abnormally arranged trabeculae with remnants of chondrocytes are formed. This disturbed organization of bone formation in the metaphyseal zone in rickets may be caused, at least partly, by the abnormal and delayed vascular invasion. The capillaries toward the cartilage form wide sinusoids, disturbing the longitudinal bars of the cartilage, which normally are not affected. The normal framework for the new formation of bone trabeculae is thus absent; therefore the bone trabeculae formed show an irregular pattern. The mineralization of the intercellular matrix of the cartilage in the epiphyseal plate, which is normally two or three cells ahead of the capillaries, can at some sites also be demonstrated in rickets.* From these results it is clear that in rickets mineral salts are deposited in the organic matrix but that the process of mineralization is very soon arrested or does not proceed in the normal manner.

In polarized light it could be demonstrated that the osteoid tissue had an organization of its collagen fibers which was different from that of the surrounding, highly mineralized bone tissue. Instead of bundles of collagen fibers running circularly in the osteons, the collagen in the osteoid tissue was often arranged in short bundles running perpendicular to the Haversian canal. X-ray diffraction of transverse sections of normal bone tissue does not give any fibering of the diffraction rings, while a good orientation is displayed by longitudinal sections. This property of the bone tissue is caused by the orientation of the collagen fibers. The observed orientation of the collagen bundles of the osteoid tissue was in full agreement with the orientation of the crystallites demonstrated in our micro x-ray diffraction diagrams and also with the findings by Clark and Mrgudich [17] that the inorganic crystalline material, though chemically unchanged, has lost its preferred orientation in the long axis of the bone.

It thus seems that in rickets there is a disturbance not only in the process of mineralization but also in the organic matrix. The osteoid tissue does not seem to be normally developed bone tissue in which absence of mineral salts is the only defect. The organic matrix of the osteoid tissue does not reach the organization which is found in normal, fully developed bone. This statement is in disagreement with the claim that there

* Hjertquist, S. O.: Personal communication.

is no reason to believe that the cells and matrices concerned in bone growth are defective in rickets (Schol [18]).

In rickets the newly formed predentin remains uncalcified for a longer period than under normal conditions. For this reason, Wilton [19] has concluded that the dentin cells in rickets do not achieve the power of producing calcifiable matrix until they have reached a point higher up in the teeth than in normal circumstances. This delayed mineralization was thought to be caused by retarded maturation of the cells responsible for the inducement of the deposition of lime salts. When, however, the odontoblasts have reached a complete differentiation, precipitation of lime salts takes place. Also, in the bone tissue the accretion of mineral salts was found to be connected with the differentiation of the cells. These statements find support from the observations made by Wolbach and Bessey,[20] viz., that the first evidence of rickets is the failure of the cartilage cells to complete the cycle of cytomorphosis, and from the evidence collected in the present investigation.

Undoubtedly, the lowered Ca × P product in serum is a factor of the utmost importance for the low mineralization of the osteoid in rickets (Shipley, Kramer, and Howland [21]). Since the work of Robison and Soames [22] and of Robison, Macleod, and Rosenheim,[23] it has also been clear that labile, probably enzymatic, reactions are connected with calcification in bone. These reactions, however, cannot be an absolute precondition for the initiation of precipitation of bone salts, as calcification may occur in vitro even if these reactions are inhibited, provided the Ca × P product is high enough (McLean [24]). Thus, the conclusion can be drawn that in rickets if the Ca × P product is low, the newly formed bone tissue cannot calcify in a normal manner until a later stage, when the tissue has reached sufficient maturation and, consequently, the intensity of the reactions necessary for calcification is culminating. Since the organic matrix formed in rickets is not normally developed, the calcification process thus becomes more inhibited than can be attributed solely to the low Ca × P product, as the matrix is not calcifiable when it is laid down.

Whether the type of matrix formed in the skeleton in rickets is evidence to the effect that vitamin D acts directly on the bone cells cannot be decided on the basis of the present study. According to investigations by Dodds and Cameron,[25] the reduction in growth which is mostly found in rickets is produced by phosphorus deficiency. Hence, it cannot be ruled out that the development of an abnormal matrix may also be caused by the deficiency.

## SUMMARY

The bone tissue in experimental rickets in dogs has been investigated with various biophysical methods. Microradiographic examination of the distribution of mineral salts has shown that in rickets there is a deposition of mineral salts in the osteoid tissue, though the mineralization is mostly very low.

In the newly formed osteoid bone tissue in rickets there is an atypical orientation of the collagen fibers, which involves an orientation of the apatite crystallites in transverse sections of the bones, as seen in the x-ray diffraction diagrams.

The changes in the growth zone of the long bones found with the methods used have been correlated to earlier results with other techniques.

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# Venous Distribution in Livers

## Comparison in Man and Experimental Animals and Application to the Morphogenesis of Cirrhosis

HANS ELIAS, Ph.D.
and
HANS POPPER, M.D., Chicago

In recent years cirrhosis has been produced in experimental animals, especially in rats and dogs.* This was mainly accomplished by exposure to poisons, to dietary injury, or to both. Several investigators † have pointed out similarity not only between experimental and human cirrhosis insofar as etiologic factors are concerned but also between experimental and human morphogenesis of cirrhosis. Such considerations are helpful, for relatively little is known about the initial stages of some forms of human cirrhosis. Recent investigations [9] concerning histogenesis of experimental cirrhosis were based on livers in which vessels had been injected. The experiments demonstrated that the trabeculae in experimental rat cirrhosis originate from the central fields and only later reach the portal canals. This observation has been confirmed by others,[6] as well as by us.[10] Hartroft,[4] moreover, has convincingly demonstrated that in rats with cirrhosis due to choline deficiency connective tissue trabeculae form from condensation of connective tissue capsules of the fatty cysts. These cysts develop first in the center of the lobules and subsequently in other areas relatively remote from termination of the portal veins.

In his observations on the rat, Hartroft [11] assumes that the portal veins in the larger portal fields do not discharge portal blood directly to the sinusoids and that, therefore, the parenchyma around the larger portal triads receives its blood from relatively remote small portal triads. That is the reason why he actually calls the parenchyma around portal triads "nonportal." Indeed, septa do reach this area, and this fact accounts for the cirrhotic dissection of the lobules.

Personal observations [10] based on injection preparations fail to indicate the primarily central character of the trabeculae in human cirrhosis; moreover, more recent morphogenetic studies,[12] based on three-dimensional analysis, indicated a mechanism other than the one described by Hartroft as being operative in the cirrhotic dissection of the lobular parenchyma, even in fatty metamorphosis. This raised the question of possible dissimilarities in the vascular distribution between human and rat liver, specifically as to whether in human beings the parenchyma around the portal triads is likewise "nonportal"—in other words, whether the parenchyma receives its blood from remote branches rather than directly from the neighboring portal triads. The vascular arborization as well as the hepatic vein distribution in the rabbit, rat, dog, and human liver was studied from this point of view. The vascular distribution in the rat liver was recently investigated by Gershbein and Elias.[13] Vascularization of the rabbit and dog liver, as described by one of us (H. E.) in 1949,[14] is accepted here and referred to as the basis for comparison with the present study.

### MATERIALS AND METHODS

Seventeen normal human livers, injected through the portal or a hepatic vein, or from both sides, were used for the present study. Twelve of them were injected with Vinylite and subsequently corroded in hydrochloric acid. The remaining five human specimens were injected with India ink,

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From the Department of Anatomy, Chicago Medical School, and the Hektoen Institute for Medical Research and the Department of Pathology of Cook County Hospital.

* References 1 through 8.

† References 1 and 4 through 6.

fixed in formol, cut with a razor into thick slices, dehydrated, cleared, and mounted in balsam. The dog and rabbit material is the same as that used in a previous study [14]; so is the material on rats.[13]

## RESULTS

### Pattern of Venous Distribution in Mammals in General

The liver parenchyma receives in general its portal vein blood from short inlet or afferent venules arising along the major course of the portal vein branches (Fig. 1, *IV*). Sinusoids emerge also from the last terminal

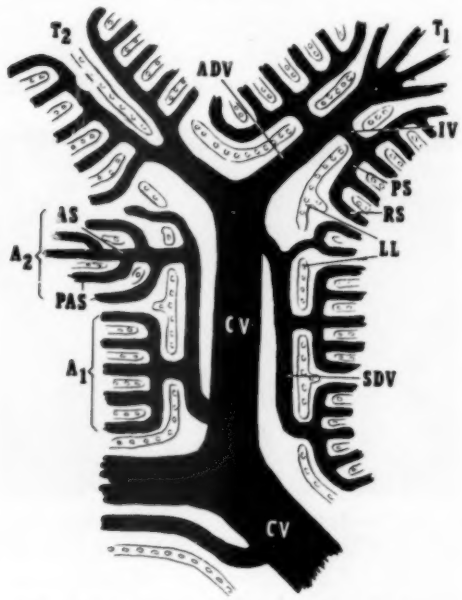



Fig. 1.—Diagram of portal vein distribution.

$A_1$ and $A_2$, indicate arborizations Type I and II; *ADV*, axial distributing vein; *AS*, axial sinusoid; *CV*, conducting vein; *IV*, inlet venule; *LL*, limiting plate; *PAS*, paraxial sinusoids; *PS*, peripheral sinusoid; *RS*, radial sinusoid; *SDV*, marginal distributing vein. $T_1$ and $T_2$, terminal twigs Type I and II. (Reprinted from Elias, H.: in Tr. of the 10th Conference on Liver Injury, New York, Josiah Macy, Jr. Foundation.)

twigs of the portal vein branches (Fig. 1, $T_1$, $T_2$). The inlet venules pierce the limiting plate; when they are inside the parenchyma, they ramify into sinusoids: periportal or paraportal, radial, and, in some cases, axial sinusoids, identified in the diagram (Fig. 1) as *PS*, *RS*, and *AS*, which occur as two types of arborization, shown as $A_1$ and $A_2$. The inlet venules may arise directly from the portal veins lying in the center of a portal canal, that is, the axial distributing veins, designated as *ADV* in Figure 1. In the case of large portal canals, however, the arrangement is different. No inlet venules arise from the veins occupying their centers. They were therefore called "conducting veins" (Fig. 1, *CV*). The supply with portal blood of the territory adjacent to these is effected by marginal distributing veins, formerly called "small distributing veins," and designated as *SDV* in the diagram (Fig. 1). These marginal distributing veins run in the same portal canals as the conducting veins. It is from these marginal distributing veins that the inlet venules arise.

The diagram (Fig. 1) serves only to illustrate the types of portal vein branches; the proportions are unavoidably distorted, for it is evident that the conducting veins must be shorter in their total combined length than the combined length of the axial distributing veins. This is a mathematical consequence of the frequently repeated dichotomous branching, for of each order of branches there are always twice as many as of the next larger order. For monopodial branching, predominance of small over large branches is equally necessary.

### Distribution of Intrahepatic Vein Branches in Different Species

*Man.*—Portal Veins:

1. Conducting veins begin with the two trunks of the portal vein and comprise the rami venae portae and their branches [15] down to a diameter of 400$\mu$. The conducting veins do not give rise to inlet venules; the territory immediately surrounding them is vascularized in a different way.

2. Marginal distributing veins arise from the conducting veins. They run alongside them (Fig. 2) in the same portal canal. These marginal veins may take several courses: They may arise from the base of a side-branch (Fig. 2, *mR, mF, M;* Fig. 3, *SC*) or from the conducting vein directly (Fig. 2, *DR;* Fig. 3, *SP, T*) and may be directed parallel to the conducting vein (Fig.

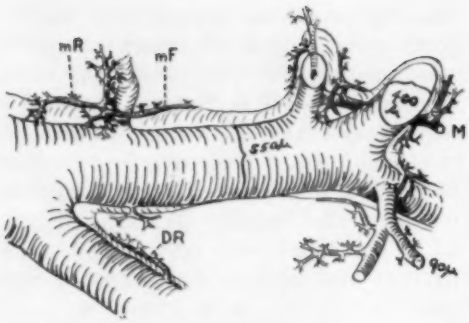



Fig. 2.—Human conducting veins and immediate surroundings, India ink-injected.

*DR* indicates marginal distributing vein arising directly from conducting vein, recurrent; *M*, marginal distributing vein arising from base of side branch and running transversely; *mF*, marginal distributing vein arising from base of side branch, blood flows forward; *mR*, marginal distributing vein arising from base of side branch, recurrent.

2, *M*); or they may surround the conducting vein in a spiral fashion (Fig. 3, *SP*), or they may take a semicircular course (Fig. 3, *SC*). The blood may flow in them in the same direction as in the larger vessel (Fig. 2, *mF*, *M*) or it may flow in a recurrent or opposite direction (Fig. 2, *mR*, *DR*; Fig. 3, *SP*).[13] From these accompanying, marginal distributing veins, inlet venules arise which supply the territory immediately adjacent to all species investigated: rabbit, rat, dog, cat, and man. The differences are only quantitative. In man, the marginal blood supply is optimal.

3. Veins intermediate between the conducting and axial distributing type are those of diameters between 280$\mu$ and 400$\mu$. They

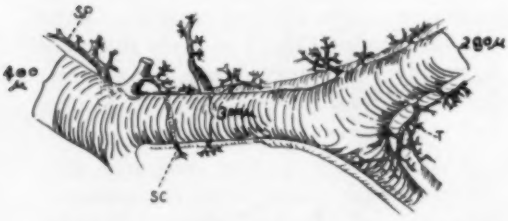



Fig. 3.—Human portal vein branches injected with India ink. Conducting vein at the left, axial distributing vein at the right, an intermediate type of vein in the middle.

*SC* indicates marginal distributing vein arising from base of side branch, running semicircularly; *SP*, marginal distributing vein arising from conducting vein, recurrent; *T*, marginal distributing vein arising from conducting vein, transverse.

give rise to both marginal distributing veins and direct inlet venules (Fig. 2, central portion).

4. Axial distributing veins are small portal vein branches from which only inlet venules arise directly. In man, portal branches of a diameter of 280$\mu$ and smaller belong to this type (Fig. 4).

*Dog.*—Portal Veins:

1. Conducting veins are the two trunks of the portal vein and the named rami venae portae and the basal part of their branches down to a diameter of approximately 250$\mu$.[14]

2. Marginal distributing veins arise from the conducting veins, have usually a spiral course, and are forward-flowing or recurrent.

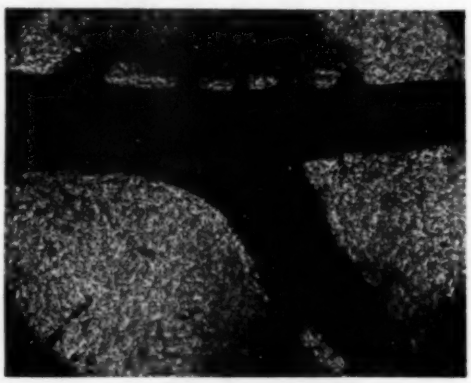


Fig. 4.—Human axial distributing veins with inlet venules and paraportal sinusoids. India ink-injected.

3. Veins intermediate between the conducting and axial distributing type, i. e., such portal branches from which both marginal distributing veins and inlet venules may arise, may be as small as 100$\mu$ in diameter.[14]

4. Axial distributing vein, i. e., those from which only inlet venules arise, may be as large as 250$\mu$ in diameter and smaller.

*Man and Dog.*—Common Characteristics of Portal Veins:

The marginal distributing veins are numerous in man and dog. They run close to the limiting plate of the large portal canals. They are so well placed that the territory surrounding the large portal canals is the best vascularized portion of the liver paren-

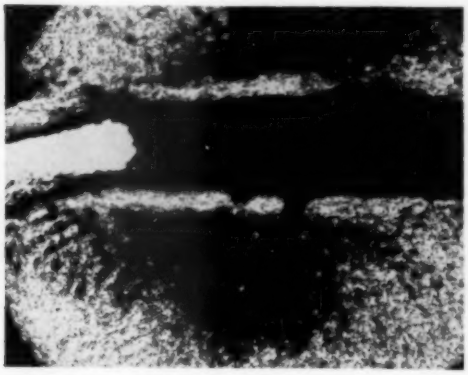
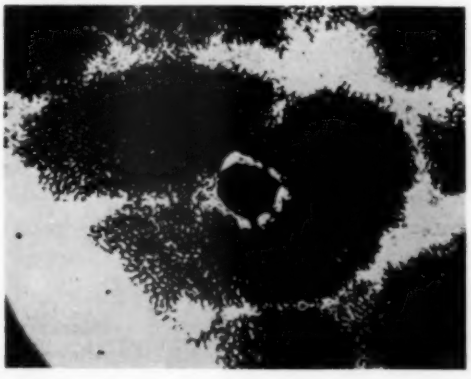


Fig. 5.—Longitudinal section of human sublobular vein. Central vein entering into it. Sinusoids entering into central vein. Note absence of sinusoid entrances into sublobular vein. India ink-injected.

chyma. India ink injected into the common portal vein will fill the immediate vicinity of the largest portal canals before it reaches areas more distant from these canals and before it reaches the lobular centers (Figs. 2, 3, and 4). All the vessels shown in Figures 2 and 3 are those actually injected. In other words, the ink had not yet penetrated to greater lobular depth than that shown in these illustrations.

The portal vascularization of the dog's liver is approximately the same as that in man, as it appears from a previous report by one of us (H. E.),[14] which was based largely on dog livers insofar as vascularization is concerned.

Fig. 6.—Cross section of human sublobular vein with four central veins entering into it. India ink-injected.



The anatomic reason for this excellent portal blood supply of the parenchyma surrounding the largest portal canals in man and dog is the large number of marginal distributing veins and their hydrodynamically convenient spacing.

*Man.*—Hepatic Veins:

One of us (H. E.) has observed[14] that in the center of the lobule an arrangement comparable to the inlet venules does not exist in any mammal. India ink-injected specimens reveal that individual sinusoids empty directly into central veins.[14] In man and dog, none were found to empty into sublobular or larger veins (Figs. 5 and 6). This means that

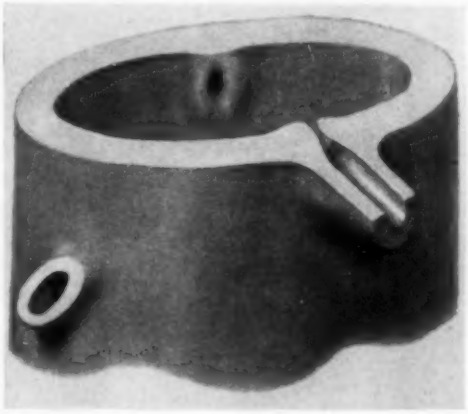


Fig. 7.—Human sublobular vein with entrances of three central veins which are narrowed while piercing through the sublobular vein. Reconstruction from serial sections.

these veins are surrounded by an uninterrupted limiting plate. For this reason drainage of human liver lobules must go through a narrow bottleneck at the centrosublobular junctions. This condition is aggravated by constrictions of the veins (Fig. 7), which results in an ampullary dilatation of the sinusoids just proximal to their entering the veins.[16] As a consequence of this arrangement, territories adjacent to sublobular and larger hepatic veins are not well drained. Also, the central veins themselves are at a disadvantage because they carry the load of draining not only their own area but also that around sublobular and larger hepatic veins.

*Dog.*—Hepatic Veins:

Dogs have a hepatic venous throttle musculature which is a system of spiral and sphincter muscles surrounding the hepatic veins from the smallest to the largest. These muscles form a partial investment of the hepatic veins and are continuous with one another. They can completely occlude the hepatic venous system (Fig. 8*A*). On longitudinal sections of sublobular veins of a dog killed with histamine these muscles appear projecting into the lumen (Fig. 8*B*), and in thick unstained sections of such a vein portions of the throttle muscles appear contracted in such a fashion as to divide the blood column into bead-like pockets (Fig. 8*A*).

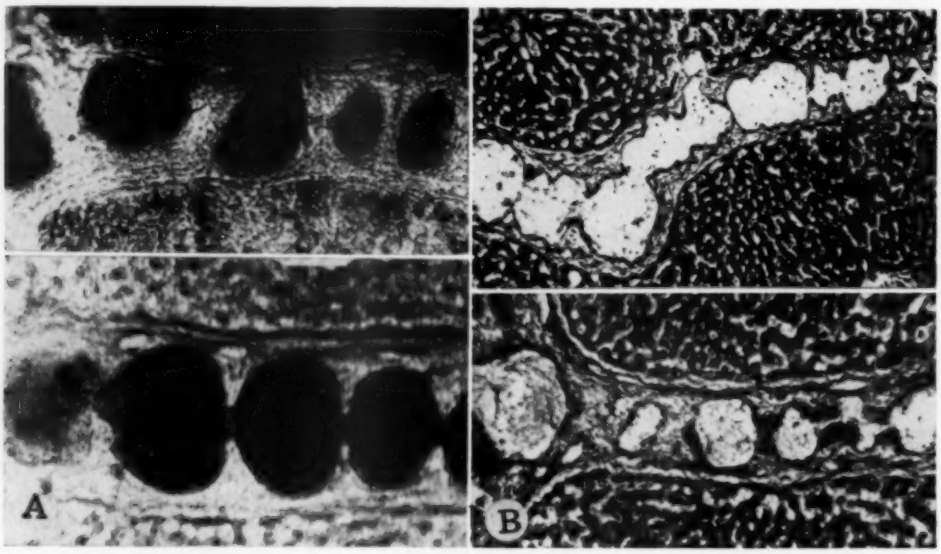



Fig. 8.—Longitudinal sections of sublobular vein of dog. *A*, thick unstained. The dark substance is blood. The mass of blood is divided in a beaded string-like fashion by the constriction of the throttle muscles. *B*, 10μ, hematoxylin and eosin. Longitudinal section of sublobular vein of dog. Contracted throttle muscles protrude into the lumen.

Large lymph vessels are found in the adventitiae of the hepatic veins of the dog which may appear wide-open (Fig. 9).

*Rat.*—Portal Veins:

In the rat, only the rami venae portae and the proximal portions of their primary branches, namely, vessels down to 200μ in diameter, are conducting veins.[18] The axial veins giving rise to inlet venules are smaller. Marginal distributing veins are relatively rare. Those present are spaced far apart [13] and arise from the base of a primary side branch and bend backward, surrounding the conducting vein in a semicircular fashion. When India ink is injected into a rat's liver, the territories in the vicinity of large portal canals are reached by the ink last. In other words, the parenchyma immediately surrounding the conducting veins in the rat is poorly vascularized, receives blood only indirectly through long stretches of sinusoids, except at the few places where a marginal distributing vein exists. Hartroft [11] was thus justified in calling this area "nonportal." To reach this region portal blood must first traverse a large area of parenchyma, being carried there through the sinusoidal network which receives blood from inlet venules that arise from axial distributing veins at least one-half lobular diameter away from the parenchyma surrounding the large portal canals.

*Rat.*—Hepatic Veins:

It has been shown [13] that individual sinusoids in the rat enter into the hepatic veins

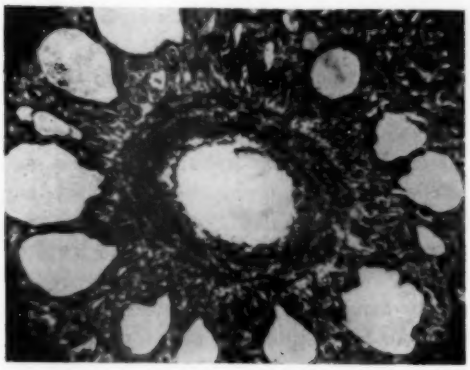


Fig. 9.—Hematoxylin and eosin. Cross section of central canal of dog, showing a portion of the throttle muscle (crescent-shaped) and lymph vessels.

at all levels, from the central veins to the largest hepatic veins. This means that all hepatic veins of the rat act as central veins and that the territory surrounding all hepatic veins is centrolobular from the standpoint of blood flow.

Direct entrances of sinusoids in close proximity to one another can be seen all along the course of these veins. In other words, the drainage of the hepatic parenchymatous continuum of the rat is homogeneous and perfectly balanced everywhere. There are no drainage bottlenecks at any level.

*Rabbit.*—Portal and Hepatic Veins:

The distribution of the portal and hepatic venous systems was previously described.[14] Vascularization from the portal side resembles that of the dog. The number of individual sinusoidal entrances into hepatic veins decreases gradually from the central to the large hepatic veins which are surrounded by a limiting plate that only rarely is pierced by a sinusoid. The limiting plate accompanies all hepatic veins down to the sublobular veins, but its perforations by sinusoids are more numerous as smaller vessels are reached. Finally, the central veins do not have a surrounding limiting plate at all.

Figure 10 summarizes the essential differences of the venous vascular pattern in man and rat.

## COMMENT

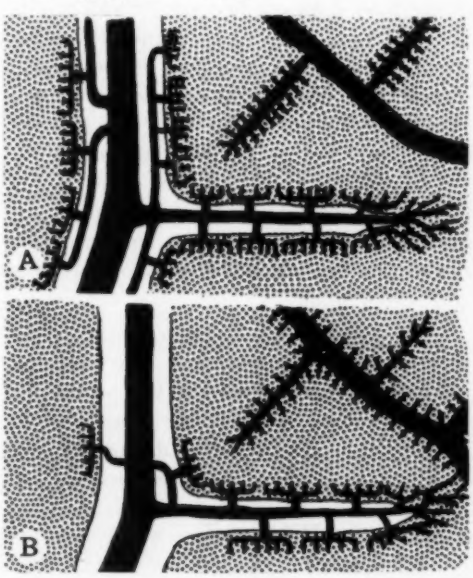
Cirrhosis is conventionally defined as an altered reconstruction of the lobular pattern with the formation of regenerative nodules.‡ The processes leading to this change are mainly collapse of massive or submassive necrotic liver tissue or the dissection of the lobules by septa,[12] both associated with the formation of anastomoses between portal and hepatic vein branches.[10] The distribution pattern of the portal and hepatic veins therefore is important in the morphogenesis of cirrhosis. The present study indicates differences in this pattern between the rat, on the one hand, and the human and dog, on the other. These differences in turn produce different pathways of lobular dissection in these species during cirrhosis formation. The observations of Hartroft [11] on the rat have been confirmed; namely, that the areas around larger portal triads receive blood

‡ References 17 through 19.

Fig. 10.—Schematic diagrams comparing venous vascularization of liver of man, *A*, with that of rat, *B*.

*A* (man): Parenchyma next to conducting vein (vertical, left) is richly supplied with portal blood through marginal distributing veins. An axial distributing vein is shown horizontal at the bottom. Sublobular vein (oblique, upper right) receives no sinusoids. Good supply, poor drainage.

*B* (rat): Parenchyma next to conducting vein (vertical, left) receives very little portal blood. Sublobular and collecting veins (oblique, upper right) receive numerous sinusoids at all levels. Poor supply, good drainage.



indirectly from relatively remote smaller portal triads and that, therefore, this area is, in the functional sense, "nonportal," similar to the central zone.

In contrast, in human beings and dogs the area around middle-sized and larger portal triads receives blood directly from the main portal branch itself. Although the main portal branch is only conducting, accompanying small branches distribute blood freely to the surrounding parenchyma. In dogs and human beings, therefore, the area around the portal triads is also, in the functional sense, portal. If one assumes, as Hartroft does,[11] that the location of maximal fat accumulation is the site of septum formation, the presence of fat in nonportal areas of the rat results in septa connecting the central zone with the larger and middle-sized portal fields. Such connecting septa subdivide the lobule. Prolonged fatty metamorphosis itself causes, therefore, cirrhosis in rats, in view of the indirect portal vein blood supply in this animal.

In human beings and dogs, however, the periportal parenchyma receives portal blood directly, and the mechanism demonstrated for septum formation and lobular dissection in the rat is not applicable to man or to dogs. In humans, prolonged fatty metamorphosis of the liver does not necessarily lead to cirrhosis.§ Two other mechanisms are operative,‖ to which the fatty liver creates a predisposition. The first is inflammation associated with hepatocellular injury and subsequent connective tissue membrane formation; the second is the development of stress fissures in the lobular parenchyma, due to uneven expansion of hepatic territories by uneven fat deposition and removal, necrosis and regeneration.

This difference in morphogenesis of the septa in different species is also reflected in their vascularization. In the process of formation of the septa in the human being, usually sinusoids are included into the septum and transformed into venous vessels. These vessels form anastomoses between branches of portal and hepatic veins which are of functional importance in the cirrhotic process.[10] They create a circulatory disadvantage for the parenchyma by shunting blood away from it and thus maintain the cirrhotic process, even when its initiating cause has disappeared. In rats with carbon tetrachloride intoxication, similar anastomoses have been described and their significance demonstrated[24]; however, they seem to be far less common in rats, for during the formation of septa in nonportal areas inclusion of vessels and sinusoids is not a significant process.

Similar to the species differences in the inflow of blood from the portal vein branches into the parenchyma, there are differences in the outflow of blood from the parenchyma to the hepatic vein tributaries. In the rat, sinusoids enter into almost all branches of the hepatic vein. In the rabbit, the draining is almost as perfect, whereas in the human being and in the dog only central veins receive sinusoids. This creates difficulties in the release of blood from the areas around sublobular and larger portal vein branches; moreover, the sinusoids entering into the central veins have to carry a compensatory heavier load. This difficulty of drainage from the lobular parenchyma is aggravated by throttle mechanisms existing in the sublobular and hepatic veins. In the dog, they are represented by the spiral and sphincter muscles. Their existence was known early[25] and they were repeatedly studied.¶ These muscles contract in shock,# and in response to histamine and other pharmacologic agents,[20] and thus cause stagnation of blood in the liver. In man, a similar but less effective throttle mechanism is produced by the arborization of hepatic vein branches; relatively small, central and sublobular veins enter large hepatic vein branches. The contraction of the larger vessels thus constricts the piercing small vessels.*

§ References 20 through 22.

‖ References 12 and 23.

¶ References 16 and 26.

# References 27 and 28.

* References 16 and 29.

The action of the sphincter mechanisms is obviously related to the regulation of outflow of blood from the liver. The sphincter mechanisms may become detrimental in aggravating the stasis in the central portion, especially around sublobular rather than central veins where the drainage is already impaired by relative difficulty in drainage of blood from the sinusoids. This difficulty in drainage can be assumed to be the reason for the frequent occurrence of central necrosis in the human being in disturbances of hepatic circulation by cardiac failure or shock. In shock as well as in toxic hepatitis, spasm of the branches of the hepatic veins occurs,† which aggravates the lesion.

The contraction of the spiral and sphincter muscles also seems to be important in lymph drainage in the dog, in which lymphatic drainage around the branches of the hepatic vein is more prominent than in other animals. The contraction of the hepatic vein opens the lymphatics, exerting a pumping effect which is especially seen in histamine shock.

The differences in the vascular distribution in the livers of man, dog, rabbit, and rat are sufficiently great to be taken into consideration in the study of the morphogenesis of hepatic alterations in these species.

## SUMMARY

The distribution of the hepatic and portal vein branches in man, dog, rabbit, and rat was reexamined and the pattern of each species compared with those in the others. New observations of the human liver were compared with the previously described distribution in the other species for application to the morphogenesis of cirrhosis and other hepatic lesions.

In the rat, in confirmation of observations by Hartroft, territories around middle-sized portal triads and larger portal triads receive their blood from portal vein branches in relatively remote smaller portal triads and are, thus, functionally nonportal. In contrast, in the human being and the dog, larger portal vein branches discharge blood into the surrounding parenchyma and numerous marginal distributing branches accompany large conducting portal vein branches; thus, the territories immediately adjacent to the portal canals, even of largest caliber, are optimally vascularized.

The dissection of the lobule by connective tissue septa formed in "nonportal" areas of maximal fat distribution in the rat cannot apply to the human being or to the dog, and other mechanisms, facilitated by the presence of fat but not caused by fat alone, are instrumental. Only for the rat does it appear proved that severe fatty metamorphosis itself produces cirrhosis.

The outflow of the blood from the hepatic sinusoids into the hepatic vein branches is more readily accomplished in rats than in man and dog. In rats, sinusoids enter freely into the hepatic veins at all levels of the hepatic venous tree. In man and dog, all sinusoids drain into the central veins alone, and none into sublobular and collecting veins. This creates a difficulty in drainage of blood which is especially severe around the sublobular and larger veins.

There is, in addition, a throttle mechanism which in the dog is muscular and in man is caused by special arborization of the hepatic venous tree. Relatively thin central veins pierce through thick-walled, firm sublobular and larger hepatic veins. Contraction of the larger veins occludes the smaller veins, which may show a sinus-like dilatation before entering. Therefore, human beings have a predisposition for centrolobular stasis and necrosis.

In view of the differences between man and rat in the distribution of the portal vein and hepatic vein branches, as well as of the lymphatics, conclusions drawn from experimental animals as to the morphogenesis of cirrhosis should be accepted with caution when applied to the human being.

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# Plexiform Neurofibromatosis of the Heart and Neck

SIMAO PUNG, M.D.†
and
EDWIN F. HIRSCH, M.D., Chicago

Neurofibromatosis is characterized by multiple mollusk-like tumors of the nerves associated with café-au-lait pigmentations of the skin. It is also designated von Recklinghausen's disease because of von Recklinghausen's classic description in 1882.[1] To differentiate the generalized osteitis fibrosa cystica, bearing the name of the same author, the term "von Recklinghausen's disease of the nervous system" is preferred. Originally, the skin tumors were called fibromas and were believed to be caused by the proliferation of the endoneural connective tissues. They are now believed to originate from the Schwann's membranes and, accordingly, have a neuroectodermal instead of mesodermal derivation.

Since the first description, an enormous literature has accumulated, either dealing with the nature of the tumors or recording the pleomorphic clinical manifestations. Recent articles with good reviews are available.* The protean manifestations of this disease are well known. Tumors of the skin, or even the café-au-lait spots, need not be present. Neurofibromatosis of the internal organs ** or of the central nervous system alone, such as the brain or the spinal cord,‡ have been recorded.

Histologically, the tumors, especially those of the skin, grow at the end of a nerve, in the form of isolated small nonencapsulated nodules, with a diffuse proliferation of the peripheral nerve elements; or the tumor tissues may grow along a nerve trunk, loosely attached to the adjacent tissues, with an edematous stroma and without appreciable proliferation of Schwann's membranes or of the supportive neuroectodermal tissues. These are known as neuromas, or plexiform neuromas.

A hereditary factor has been well established in that the disease may occur at a young age and more than one member of a family may be affected.[9] The frequent association of neurofibromatosis with various other disorders supports the view that this is a systemic disease. Congenital malformations [10] and tumors of various parts of the body § have been reported frequently. Many believe that pheochromocytoma of the suprarenal gland is related to the neurofibromatosis or to the other neurocutaneous syndromes, such as hemangiomatosis (von Hippel-Lindau disease), tuberous sclerosis, and Sturge-Weber syndrome. However, among the visceral organs, the heart is rarely involved. The primary location in the pharynx with symptoms of obstruction of the upper respiratory tract is also uncommon. As far as we are aware, no involvement of the heart has been found, and only three cases of neurofibroma of the pharynx associated with neurofibromatosis have been recorded.

## REPORT OF A CASE

A Negro girl, aged 3 years 11 months, was admitted to St. Luke's Hospital, Chicago, on June 1, 1953, under the care of Dr. P. Holinger. The child

From the Henry Baird Favill Laboratory of St. Luke's Hospital.

† Dr. Pung died Oct. 30, 1954.

* References 2 through 4.

** References 5 and 6.

‡ References 7 and 8.

§ References 11 and 12.

Fig. 1.—The heart, illustrating the neurofibrous tissues encircling the base of the ventricles (*A*) and the junction of the cavae (*B*) at the upper level of the right auricle.

had a tracheotomy at the age of 2 months because of symptoms of tracheal obstruction. This had closed but was reopened at the age of 8 months because of recurrent respiratory embarrassment. She was first admitted to St. Luke's Hospital in January, 1953, after a trace of blood had been coughed up through the tracheotomy tube. Bronchoscopy was done. A mass in the posterior commissure of the larynx was found, apparently in connection with the right arytenoid. A biopsy of this mass led to a diagnosis of edematous submucosal neurofibroma. Fluoroscopic examination of the chest revealed a markedly enlarged heart.

The second admission, in March, 1953, was because of fever. Antibiotics were given, and the temperature subsided. Roentgen examinations of the chest again showed a huge heart, with atelectasis of the left lung. An electrocardiogram tracing showed a sinus rhythm, with premature ventricular systoles, periods of paroxysmal ventricular tachycardia, inverted T waves in leads $tV_3$ and $tV_4$ and a right axis deviation. The P-R interval was 0.16 second and the Q-S complex 0.07. The heart rate was 95 per minute.

The final admission, on June 1, 1953, was because of sudden onset of labored respirations. The temperature was 100.4 F. The pulse rate was 130, and the respirations were 36 per minute. The child was in severe respiratory distress. The breath sounds in the left lung were diminished. There were no other unusual physical findings. The blood had 4,250,000 red blood cells per cubic millimeter, with 9.9 gm. of hemoglobin per 100 cc., and 25,000 white blood cells per cubic millimeter, of which 77 were neutrophiles, 18 were lymphocytes, 2 were monocytes, and 3 were band forms, per 100 white cells counted.

During the final admission the patient was bronchoscoped several times; the left and right main bronchi were narrowed, and a large amount of thick, tenacious, purulent secretion was aspirated from each side. The left lung was atelectatic. Cultures of this secretion yielded a moderate growth of Staphylococcus aureus. On June 12, an erythematous, morbilliform rash appeared on the face and the trunk. This was diagnosed as rubeola. In the morning of June 22, the respirations became shallow and labored; the child was cyanotic, and death occurred shortly thereafter.

*Necropsy.*—A necropsy of the trunk was made approximately five hours after death.

The body weighed 45 lb. (20.4 kg.) and had a crown-heel length of 99 cm. and a crown-rump length of 54 cm. The frontal bone of the calvarium was flattened, so that the anterior-posterior diameter of the head was much smaller than the transverse. The left pleural space contained 300 cc. of a clear yellow fluid, and the entire left lung was markedly atelectatic. The right lung was expanded. The left lung weighed 180 gm., and the right, 320 gm.

When the pericardial sac was opened, a markedly misshapen heart was exposed. Where the superior and inferior venae cavae joined to enter the right auricle, there was a ring-shaped mass of dense fibrous tissue, with an outside diameter of 6.5 cm. and shaped like a doughnut. It ranged in thickness between 2 and 3 cm. and was hard, elastic, and pearly white. The auricle with the auricular appendage formed a zone of cardiac tissue about 5 cm. wide and then merged at the level of the tricuspid ring into another much larger ring of dense white fibrous tissue with an outside diameter of 7 cm. and 4.5 cm. wide. This fibrous ring merged into the ventricle portion of the heart. In the region of the pulmonary artery and the aorta was another circular mass, like a collar of fibrous tissue, with an outside diameter of 7.5 cm., and it ranged in width between 2.5 and 4 cm. The right and left ventricle tissues of the heart below these two collars were cone-shaped, and to the apex were 6.5 cm. long and at the base were 8.5 by 8 cm. The wide rings of dense fibrous tissues around the pulmonary artery and the aorta were continuous around the heart at the mitral ring level. The ring of dense fibrous tissues about the lower portion of the right auricle extended around the heart at the level of the mitral ring and about the root of the pulmonary artery. Above this was the ring first mentioned, at the confluence of the superior and inferior cavae (Fig. 1). The heart weighed 320 gm.

The subcutaneous tissues of the neck had no significant changes. There were no anomalies of the great vessels. On the right side of the neck was a bundle of edematous gray, fibrous cords that extended along the course of the right common carotid artery (Fig. 2[2]). Traced upward, these

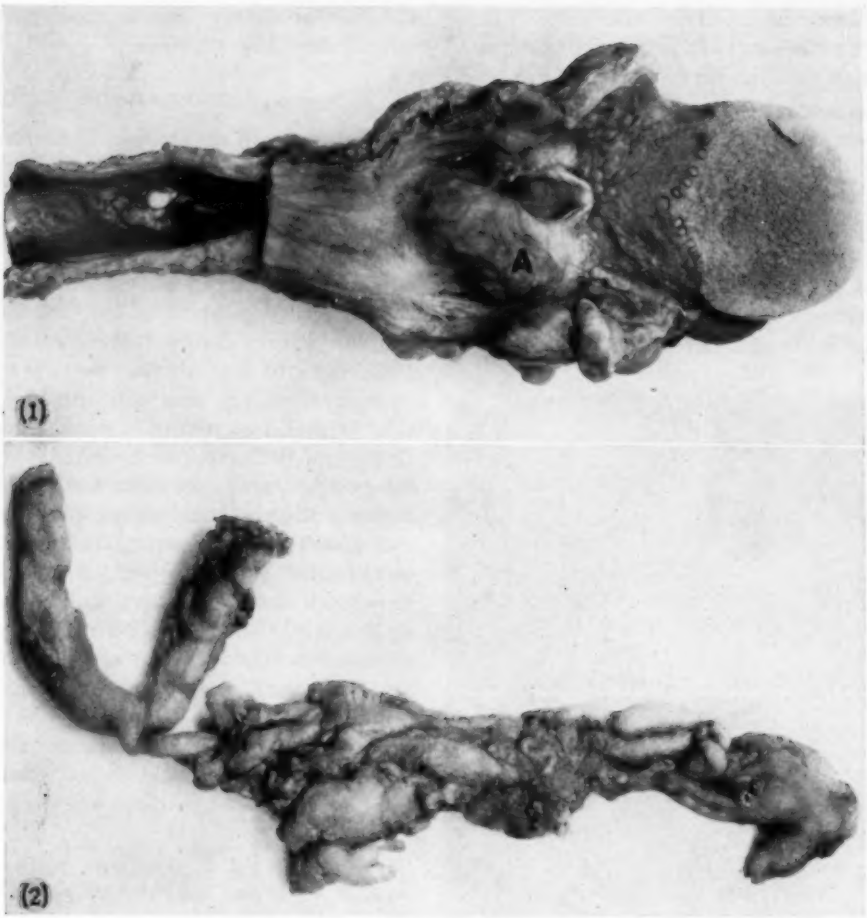


Fig. 2.—(1) The tongue, hypopharynx, aditus, and upper portion of the trachea with tracheotomy, and (2) the neurofibrous cords of tissues removed from the right side of the neck. Note in (1) the submucous neurofibrous nodule (*A*) on the right side of the aditus. A biopsy of this established the clinical diagnosis of neurofibromatosis.

seemed to end in the region between the base of the tongue and the cricoid cartilage. Distally they spread into the region posterior to the heart and seemed to be lost in the surrounding tissues.

The trachea was opened from behind. The lining of the anterior wall in the region of the tracheotomy opening and below it was granular for a region of 3 cm. In the region of the hypopharynx, just to the right of the epiglottis, was a gray mass 3 by 1.5 cm. almost completely occluding the lumen (Fig. 2*A*).

*Microscopic Examination.* — Sections of the nodule of the pharynx had a narrow layer of surface squamous epithelium. The stroma was markedly edematous and had many round structures, with concentrically arranged fibrils like edematous nerve trunks. Portions near the center of some of these trunks were more compact. Some of these trunks were between the adjacent skeletal muscle bundles.

Sections of the fibrous tissues of the heart had in the subepicardial fat many edematous nerve trunks (Fig. 3*B*), with concentrically arranged fibrils like those described. The nerve fibrils extended into the muscle tissues of the myocardium, and the muscle fibers were separated by the bundles of these fibrils. There was also some proliferation of fibrous connective tissues. These details were emphasized in sections stained with phosphotungstic

acid-hematoxylin. The dark purple muscle fibers of the heart in these sections contrasted sharply with the nervous tissues.

Sections of the cords of the right side of the neck (Fig. 3*A*) had an edematous capsule. The stroma was markedly edematous. In the center were a few nerve fibers more compactly arranged, and toward the periphery the nerve fibers were widely separated. Here and there were a few ganglion cells.

Sections of the suprarenal glands had a thin fibrous capsule. The cells of the cortex

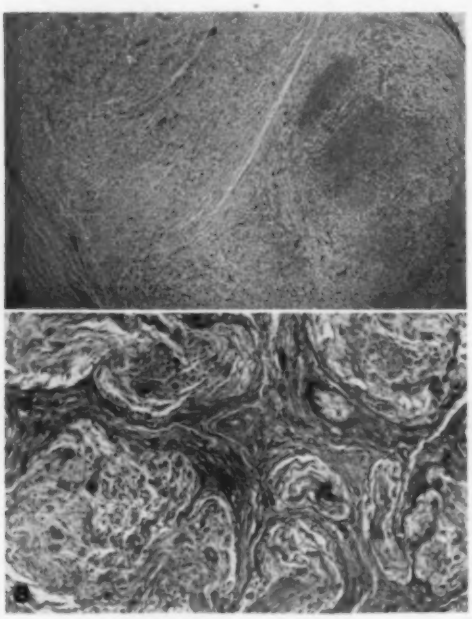

Fig. 3.—Photomicrographs: *A* (low-power), the structure of the neurofibrous cords in the neck; reduced ⅙. *B*, the neurofibroma tissues of the heart; reduced ⅓ from mag. × 198.

were arranged in the usual zona glomerulosa, zona fasciculata, and zona reticularis. Only a small amount of lipid was in the cytoplasm of the cortical cells. In the medulla were a few chromaffin cells. At one level were many ganglion cells, with pale vesicular nuclei and a slightly basophilic granular cytoplasm. These cells did not resemble the adjacent chromaffin cells, which had the typical dark granules in the cytoplasm. Extending from these ganglion cells were a few nerve fibers. Sections stained with Sevki's Giemsa method showed that the cytoplasm of these ganglion cells contained no granules.[13]

## COMMENT

Involvement of the pharynx in neurofibromatosis is infrequent. Forbes[14] in 1925 reported a plexiform neuroma associated with von Recklinghausen's disease in a girl aged 14 years. The tumor occupied practically all of the faucial pharynx and extended into the right tonsillar pillar. The left half of the pharynx was not involved. Meeker,[15] also in 1925, reported a plexiform neuroma of the pharyngeal mucous membrane. She was unable to find any record of similar tumor. Davis[16] in 1935 reported a neurofibroma of the pharynx associated with von Recklinghausen's disease in a 44-year-old woman, and considered this unique. The tumor was encapsulated and occupied the entire left pharyngeal fossa, bulging almost to the median line of the pharynx. In recent years solitary neurofibromas of the pharynx have been reported.‖ None was associated with neurofibromatosis.

Neurofibromatosis of the larynx is also rare. In 1950 Holinger and Cohen[20] reported one case and stated that only one had been reported.

The growth of a plexiform neuroma is usually centripetal, and death results from the involvement of the spinal cord or the brain. Involvement of the heart is unusual. Nieberle and Cohrs in 1931 described multiple diffuse neurofibromas of the axillary plexus, sympathetic nerve, and heart in the ox.¶ No such case has been reported in man.

The presence of ganglion cells in the suprarenal gland probably represents an abnormal development of the sympathetic nerve tissues. The association of pheochromocytoma with neurofibromatosis or other neurocutaneous disorders has been reported.# The relations between them, however, remain obscure. It probably has no more significance than

‖ References 17 through 19.

¶ Nieberle, K., and Cohrs, P.,[21] cited by Willis, R. A.: Pathology of Tumors, 2d Ed., St. Louis, C. V. Mosby Company, 1953.

# References 23 through 28.

diverse other coexistent pathological conditions, like a neurocytoma together with a neurofibrosarcoma, or other conditions.*

Since neurofibromatosis is frequently associated with various developmental abnormalities and tumors, Inglis [31] in 1950 advanced the hypothesis of a basic intrinsic factor and a neural intrinsic factor. He suggested that, if the disturbance lies at the basic intrinsic factor level, bone lesions and congenital malformations occur, and, if the disturbance happens to be at the neural intrinsic factor level, the disorders such as cutaneous pigmentation, lipoma, fibroma, etc., occur.

SUMMARY

Neurofibromatosis in a Negro girl aged 3 years 11 months is described. The pharynx and the heart were involved. Only three cases of plexiform neurofibroma of the pharynx associated with neurofibromatosis have been reported. No involvement of the heart in neurofibromatosis apparently has been reported in human beings. The presence of ganglion cells in the suprarenal glands is considered incidental with the neurofibromatosis. The etiology of neurofibromatosis remains obscure.

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# News and Comment

## ANNOUNCEMENTS

**Course in Cancer Detection.**—A course in exfoliative cytology, to be given March 7 to June 3 at Cornell University Medical College (1300 York Ave., New York), is open to a limited number of physicians and technicians. Arrangements for shorter periods of instruction covering certain applications of exfoliative cytology may be made for those who are unable to take the entire three-month course. Tuition for the course is $300, or a proportionate fee for shorter periods. Instruction, under the direction of Drs. George N. Papanicolaou and John F. Seybolt, will include laboratory procedures and will cover the cytology of the female genital, gastrointestinal, respiratory, and urinary tracts, as well as exudates.

**Fellowship in Dermal Pathology.**—The Earl D. Osborne Fellowship, sponsored by the American Academy of Dermatology and Syphilology, will provide annually the opportunity for training in dermal pathology to a postgraduate student who has completed at least one year of training in dermatology. The stipend is $4000 a year, divided into four quarterly payments during the year's fellowship. The period of training will be spent at the Armed Forces Institute of Pathology, Washington, D. C., the appointment being subject to approval by the director of the institute. The student must complete one year of graduate training, either before or after completion of the Osborne fellowship, in an institution approved by the board for three years of training. Application blanks may be obtained from Dr. Hamilton Montgomery, Chairman, Committee on Pathology, American Academy of Dermatology and Syphilology, 200 First St. Southwest, Rochester, Minn. The next available appointment begins July 1.

**Meeting of American Goiter Association.**—The American Goiter Association will hold its annual meeting in the Skirvin Hotel, Oklahoma City, April 28, 29, and 30, 1955. The meeting will consist of papers and discussions dealing with the physiology and diseases of the thyroid gland.

**Bertner Foundation Award and Lecture.**—On March 11, 1955, Dr. George N. Papanicolaou received the fifth annual Bertner Foundation Award at The University of Texas M. D. Anderson Hospital and Tumor Institute. At this time he gave the Bertner Lecture at the Ninth Annual Symposium on Fundamental Cancer Research.

**Annual Meeting of Blood Banks Association.**—The Eighth Annual Meeting of the American Association of Blood Banks will be held at the Palmer House, Chicago, Nov. 19-21, 1955. For further information, write to the American Association of Blood Banks, 725 Doctors Building, 3707 Gaston Ave., Dallas 10, Texas.

# Histochemical Studies of Early Experimental Myocardial Infarction

## Periodic Acid-Schiff Method

HISAKO O. YOKOYAMA, Ph.D.
ROBERT B. JENNINGS, M.D.
GEORGE F. CLABAUGH, M.D.
and
WILLIAM B. WARTMAN, M.D., Chicago

The detection of histologic changes in very early myocardial infarcts is generally recognized to be difficult. For example, one-half hour after ligation of a coronary artery the myocardial fibers may show only edema and indistinct changes in striation,[1] whereas definite changes usually appear after several hours of ischemia.[2] However, quantitative chemical determinations show that even a brief period of vascular occlusion causes marked changes in the chemical constituents of the myocardium.* The investigation reported here represents a systematic study of the histochemical changes that occur in early myocardial infarction. Tests for polysaccharides, protein, enzymes, lipids, and inorganic constituents were made on ischemic and normal tissues, as were observations with the phase-contrast and polarizing microscopes.[6] The results obtained by the periodic acid-Schiff reaction will be reported at this time.

### MATERIALS AND METHODS

Twenty-seven adult mongrel dogs of both sexes, and one puppy, were used as control and experimental animals. They were fed Borden's dog meal and allowed water.

Myocardial infarcts of predictable location were produced in the left ventricle by ligating a branch of the left coronary artery, according to procedures previously established.† The dogs were anesthetized with an intravenous injection of 1 ml. of 60 mg/cc. sodium pentobarbital (Nembutal) per 5 lb. (2.25 kg.) of body weight. Positive intra-alveolar oxygen was applied during surgery, and aseptic conditions were employed when a postoperative period of six hours or longer was desired. An incision was made in the left fifth intercostal space, and the pericardium was opened. In 17 animals the anterior descending ramus of the left coronary artery and the accompanying veins were ligated just proximal to the apical branch.‡ This resulted in predominantly subendocardial infarcts that were consistently located in the anterior apical region of the left ventricle. In seven animals the ligature included only the apical branch and its concomitant veins, thus producing an infarct in the same place, but of smaller size.

Samples of ischemic myocardium were collected at the following intervals after ligation: 20 to 25 minutes; 1, 2, 3, 4, 5, and 6 hours, and 1, 2, 4, 7, 14, 28, and 42 days. Since chief interest centered around the early changes, two to four animals were used in each interval up to four hours. For each of the succeeding periods, one animal was used. The animals were killed either by air embolism or by exsanguination following pentobarbital anesthesia. The heart was excised immediately, and samples of both affected and unaffected tissues were taken from the left ventricle. The unaffected (control) tissues were taken from the anterior wall above the ligature or from the posterior wall adjacent to the septum. For additional controls four normal hearts were used. Two were from dogs (Dogs 21 and 22)

From the Department of Pathology, Northwestern University Medical School.

Presented at the 39th meeting of the American Society for Experimental Pathology, Atlantic City, N. J., April 16, 1954.

This work was partly aided by grants from the Chicago Heart Association and the American Heart Association.

* References 2-5.

† References 7 and Wartman, W. B.; Campbell, L., and Craig, R. L.: The Effect of ACTH on Experimental Myocardial Infarction in Dogs, unpublished data.

‡ Wartman, W. B.; Campbell, L., and Craig, R. L.: The Effect of ACTH on Experimental Myocardial Infarction in Dogs, unpublished data.

subjected to anesthesia and surgery in the usual manner except that ligation was omitted. They were killed one-half hour and three hours, respectively, after operation. The other two hearts were from Dogs 23 and 24, which were anesthetized but not subjected to sham operation. These dogs were killed by air embolism, and tissues from the left ventricle were used as untreated controls.

Hearts from Dogs 23 and 24 were also used to study the histochemical changes taking place during autolysis. For this purpose the tissue was placed in closed moist containers at 27 C, and samples were collected at intervals of one, three, and five hours. A similar study was performed on control and ischemic tissues from Dogs 35 and 37, which had infarcts of one hour's duration.

The freezing-drying technique was employed as the principal method for preservation and localization of water-soluble substances. Several representative pieces less than 0.5 mm. in thickness were taken from all control and experimental tissues. These were spread on aluminum foil, frozen in isopentane chilled with liquid nitrogen, and dehydrated in a simple apparatus at — 30 C.[8] Tissues were also fixed in 10% buffered neutral formalin and in chilled absolute acetone. In some instances samples were fixed in Maximow's Zenker-formalin for 3 hours and in chilled alcoholic formalin (10% formalin in absolute alcohol) for 24 hours.

Studies on glycogen and other polysaccharides were made on tissues prepared by freezing-drying or fixed in 10% buffered formalin or chilled alcoholic formalin. Most of these studies were performed on frozen-dried material which was cut serially at 6μ and mounted two sections per albuminized slide. The slides were kept cool (5 to 8 C) and dry and were used within a few days.

In order better to correlate the histochemical and optical changes in any sample under examination, the slides were treated in the following manner: (1) Schiff reaction without prior oxidation, (2) α-amylase treatment followed by periodic acid-Schiff reaction, (3) periodic acid-Schiff reaction, (4) hematoxylin and eosin, (5) alkaline phosphatase reaction, (6) control for alkaline phosphatase reaction, (7) unstained section for examination with phase-contrast and polarizing microscopes, (8) Heidenhain's connective tissue stain, and (9) microincineration for inorganic constituents. By comparing results from these different procedures it was possible to correlate changes in injured tissue much more readily than could be done otherwise. Tissues fixed in formalin and alcoholic formalin were also studied, with use of serial sections 7μ thick whenever possible.

Glycogen was demonstrated by the periodic acid-Schiff (PAS) technique of McManus,[9] with and without hematoxylin counterstaining. Control slides were prepared by digestion of glycogen with 0.1 % to 0.2% α-amylase § for one-half to one hour at room temperature, depending upon the thickness of the section, the glycogen content, and the fixative employed. A 0.2% solution effectively removed glycogen from myocardium fixed in either alcoholic formalin or formalin, without altering the cytologic details of nuclei and cytoplasm. These slides were compared with those treated with three changes of saliva for one-half hour. Additional control slides were prepared without prior oxidation to determine the presence of naturally occurring Schiff-positive substances. Acetylation was performed according to the method of McManus and Cason.[10] The allochrome procedure of Lillie [11] was also applied to some samples. Unstained tissues were examined with the phase-contrast and polarizing microscopes for additional information on the physical changes occurring in the injured fibers.

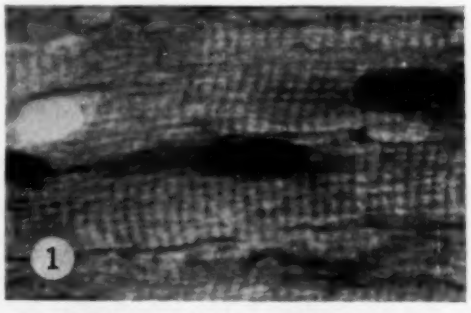



Fig. 1.—Frozen-dried normal myocardium treated with the PAS method and counterstained with hematoxylin. The nuclei stain with hematoxylin and appear dark. The sarcolemma, Z discs, and intercalated discs show a positive periodic acid-Schiff (PAS) reaction but do not contain glycogen, since they resist digestion with saliva or α-amylase. Sarcoplasm, which does contain glycogen, appears as delicate, darkly stained streaks running the length of the myocardial fibers. The myofibrils lie between the streaks of sarcoplasm and do not stain. Note the increased concentration of glycogen at the Z discs and around some nuclei. The perinuclear PAS-positive reaction is due partly to glycogen and partly to lipochrome pigment; × 560.

Samples of liver, kidney, spleen, lung, and adrenal were fixed in 10% buffered formalin for routine examination. No significant lesions were noted except in the kidney, which showed chronic interstitial nephritis in 14 of the 28 dogs. Of the three animals used for the 20- to 25-minute group, one showed numerous small groups of calcified necrotic myocardial fibers in the left ventricle, and therefore this animal was discarded.

§ Obtained from Nutritional Biochemicals Corporation, Cleveland.

RESULTS

*Observations on Glycogen.*—Frozen-dried tissues from both normal and injured areas give the most satisfactory results for the localization of glycogen, and therefore the following descriptions are based on frozen-dried material.

The normal myocardial fibers of the left ventricle contain stainable glycogen that appears as very fine particles or as a reticulum in the sarcoplasm. The staining reaction with PAS is distinctly lighter than in the liver, which contains considerably more glycogen than the myocardium. When the concentration of glycogen is low, a distinct aggregation occurs in the sarcoplasm on either side of the Z disc; when the concentration of glycogen is high, the aggregations at the Z discs are more prominent and the sarcoplasm appears as intensely stained parallel streaks between the myofibrils (Fig. 1). A high concentration of glycogen may also occur in the perinuclear sarcoplasm. Purkinje fibers contain considerably larger amounts of glycogen than myocardial fibers (Fig. 2).

After the onset of ischemia, a marked reduction in glycogen content soon occurs. The first recognizable change is a mottled appearance of the sections due to loss of glycogen in individual fibers or small groups of fibers. This diminution gradually extends to larger areas of the occluded region, and within one hour a marked contrast is observed between fibers that have been affected by ischemia and those that presumably are unaffected (Fig. 2). Extensive areas become completely negative for stainable glycogen as the duration of circulatory arrest is increased. In contrast, the Purkinje fibers retain most of their glycogen.

In the areas where the glycogen is reduced the hematoxylin-and-eosin stain shows an early disorganization of the fibers (Figs. 3 and 4). This change is exaggerated in frozen-dried material by the formation of visible ice crystal artefacts due to increased water content in the damaged areas.[4] Structural disorganization is also readily observed with the phase-contrast and polarizing microscopes.

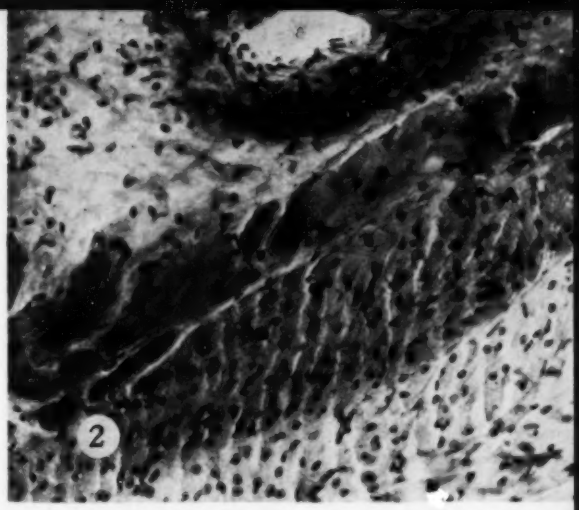
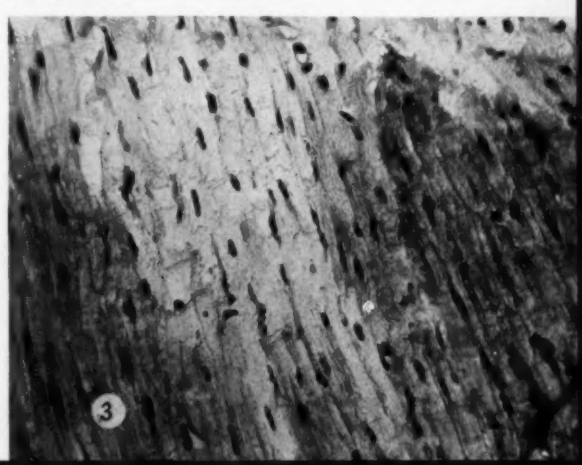



Fig. 2.—Frozen-dried myocardium showing differences in the glycogen content of Purkinje fibers and normal and ischemic myocardium. A large blood vessel, partly surrounded by connective tissue, occupies the upper part of the picture, and below the vessel are three zones of tissue. The one nearest to the blood vessel consists of Purkinje fibers, rich in glycogen. The middle zone is composed of myocardium containing the normal amount of glycogen. The outermost zone shows the loss of stainable glycogen after one hour of ischemia. PAS reaction counterstained with hematoxylin; × 190.

Some fibers surrounding the infarct begin to show more than normal amounts of glycogen several days after operation, when the necrotic fibers are being replaced by granulation tissue. This has been observed up to 42 days. The fibers adjacent to the scar tissue usually have larger diameters than normal fibers.

The same changes are observed in tissues fixed in chilled alcoholic formalin, but in these preparations the glycogen is more coarsely aggregated than in frozen-dried tissues and fixation artefacts are evident. Nevertheless, these preparations are defi-

Fig. 3.—Frozen-dried myocardium from an ischemic area six hours old. The fibers in the center and upper left hand part of the photograph show loss of glycogen and edema. PAS reaction counterstained with hematoxylin; reduced 20% from mag. × 288.



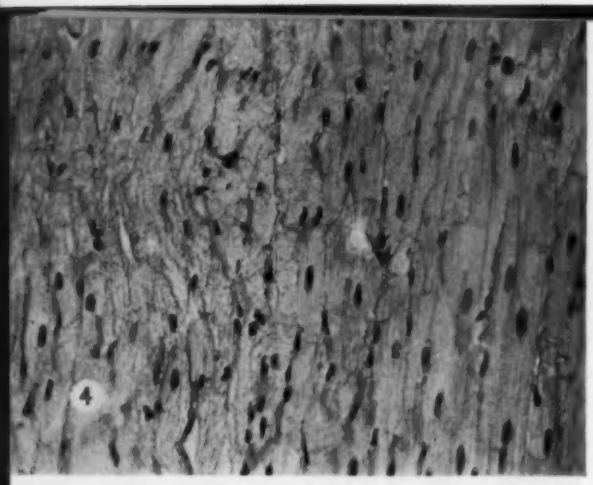
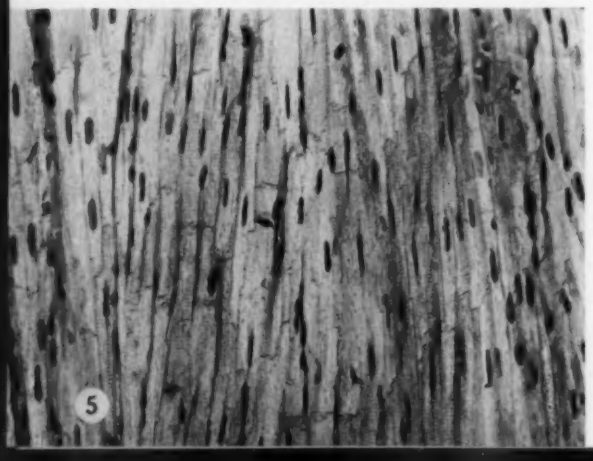


Fig. 4.—An area similar to the one shown in Fig. 3 but taken from an adjacent section, which was pretreated with saliva before the PAS reaction. The injured area is on the left hand side. Note the removal of glycogen from the normal myocardium and the marked edema and ice crystal artefact in the injured myocardium. Counterstained with hematoxylin; reduced 20% from mag. × 288.

nitely superior to those fixed by aqueous formalin, in which there is frequently gross displacement of glycogen toward one side of the fibers or against the intercalated discs. Thus, it is difficult to observe the delicate changes occurring in the very early stages of ischemia in tissues prepared by chemical fixation.

*Observations on Glycogen During Autolysis.*—Frozen-dried tissues of normal ventricle collected at intervals of one, three, and five hours after excision of the heart show a progressive loss of stainable glycogen. The sequence of changes is similar to that seen in ischemic injury. At one hour, with the PAS technique, the myocardium appears mottled owing to loss of stainable glycogen in individual as well as in groups of fibers. The degree of change varies among different fibers. The extent and amount of loss increase at three hours (Fig. 5), and by five hours most of the fibers have lost all stainable glycogen. Tissues prepared by chemical fixation show a similar sequence of changes.

Fig. 5.—Frozen-dried normal myocardium taken from a three-hour postmortem sample to illustrate the effect of autolysis. Some fibers show a reduction in glycogen. PAS reaction counterstained with hematoxylin; reduced 20% from mag. × 288.



*Observations on a Degenerative Change in the Myocardial Fibers.*—In addition to the reduction in glycogen content, another reaction to ischemia occurs that is believed to be a degenerative process. This lesion consists of swelling disorganization and intense diffuse staining with PAS of the myocardial fibers. This PAS-positive material is not glycogen, since it is unaffected by digestion with α-amylase or saliva and appears diffusely distributed through the fiber, unlike glycogen, which is limited to the sarcoplasm (Figs. 6 and 7). This PAS-positive material is not observed in the 20- to 25-minute samples; however, after one to two hours some of the injured fibers exhibit a homogeneous pink staining. Other fibers, although structurally altered, do not show this staining reaction. Samples from the 3- to 6-hour groups are definitely PAS-positive, but the intensity of reaction is still somewhat variable (Fig. 6). By the end of 24 hours all necrotic fibers become bright reddish-purple (Fig. 7) and remain so through the 14th day but have disappeared by the 28th day. These changes are most marked in chemically fixed tissues. In frozen-dried tissues a distinct PAS reaction is not observed in the degenerating fibers in very early infarcts. In one-day-old infarcts, however, a diffuse pink color is apparent in the necrotic fibers. Observations with the phase-contrast microscope reveal that when the fibers begin to show this diffuse PAS reaction the A and I bands are already partially or fully disorganized.

The exact nature of the positive PAS reaction in the degenerating myocardial fibers is unknown, but the following information has been obtained. Acetylation blocks the reaction, and treatment with 0.1 N KOH for 15 minutes after acetylation followed by the PAS reaction restores the color of the reactive materials.

Myocardium undergoing postmortem autolysis begins to show a similar slight pink stain after three hours at 27 C, and the color becomes more distinct at five hours. This change also occurs in infarcts, for when tissue from infarcts one hour old is allowed to undergo autolysis the ischemic fibers show an accentuated reaction and are thus darker than adjacent nonischemic myocardial fibers.

*Observations on Other PAS-Positive Materials.*—The following structures normally show a strong positive reaction with the periodic acid-Schiff method after digestion of glycogen with α-amylase or saliva: periendothelial basement membrane, lipochrome granules, sarcolemma, Z disc, intercalated disc, ground substance, and fibrillar connective tissues. A moderate to strong reaction is also observed in the plasma. The elastic tissue shows a positive Schiff reaction without prior oxidation, as has been observed by others.[12]

The PAS-positive materials other than glycogen are more readily observed in chemically fixed tissues than in frozen-dried material. Lipochrome granules stain only faintly after freezing-drying but are bright brownish-red after formalin or alcoholic formalin fixation. They retain the reddish color after the allochrome procedure. The sarcolemma stains intensely with the PAS method and is continuous with the Z discs and intercalated discs. After the allochrome method the sarcolemma and Z discs are purplish-red but the intercalated discs are dull greenish-blue, probably because of superimposition of stains. Closely applied to the outer surface of the sarcolemma are thin fibrils of bright blue collagen.

The appearance of the intercalated discs varies somewhat with the fixative employed. After alcoholic formalin they often are homogeneous ribbon-like bands, but after formalin or Zenker-formalin their outlines are irregular and they resemble an interwoven meshwork embedded in a homogeneous matrix. In chemically fixed tissue intercalated discs form barriers against which the sarcoplasmic constituent, glycogen, accumulates. Very abrupt differences in lipid staining have also been observed in limited areas of fibers bordered by the intercalated discs.‖

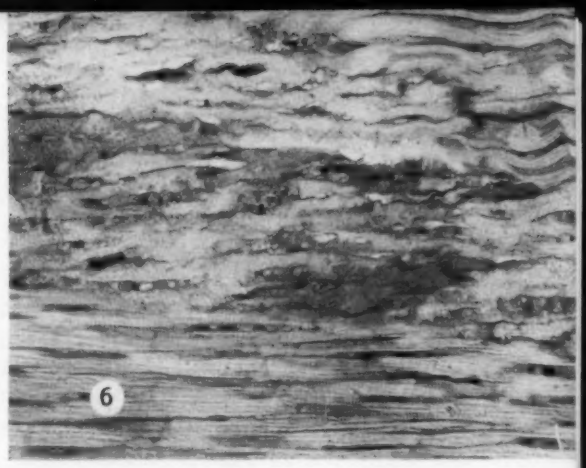


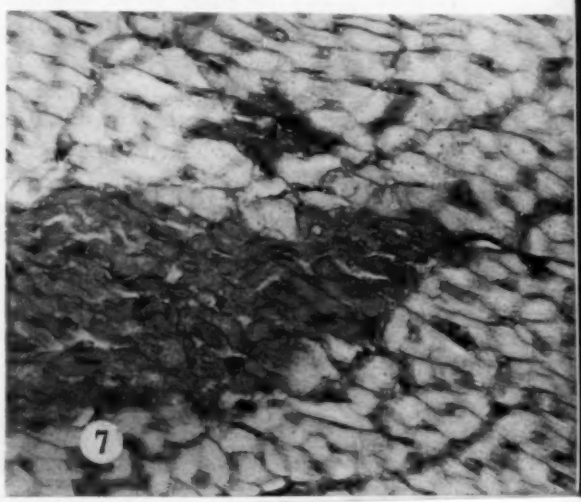
Fig. 6.—Formalin-fixed myocardium from an area affected by four hours of ischemia. Section was treated with α-amylase and stained by the PAS method. No counterstain was used. The injured fibers are disorganized, and some of them show a strong positive reaction; reduced 20% from mag. × 288.

## COMMENT

The present study has demonstrated that histochemical methods can reveal distinct changes in the dog myocardium as early as one hour after occlusion of the coronary vessels. The use of the freezing-drying technique and the sensitive periodic acid-Schiff method for the detection of glycogen and other substances containing the 1:2-glycol grouping (or the equivalent amino or alkylamino derivatives) make it possible to distinguish ischemic myocardial fibers soon after experi-

‖ Yokoyama, H. O.; Jennings, R. B.; Wartman, W. B., and Clabaugh, G. F.: Unpublished data.

Fig. 7.—Formalin-fixed myocardium from an area affected by 24 hours of ischemia. Section was treated with α-amylase and stained by the PAS method. No counterstain was used. Oblique section showing the strong diffuse reaction in groups of degenerating fibers; × 288.



mental vascular occlusion. A decrease of stainable glycogen in some groups of fibers by one hour indicates a rapid utilization of glycogen under anaerobic conditions. As the glycogen becomes depleted, some of the fibers begin to show, in addition, a homogeneous PAS-positive reaction which is characteristic of fibers undergoing degeneration.

These early changes in the ischemic areas are patchy. Not all fibers are affected simultaneously, and with time progressively larger areas become necrotic. This is probably due to variation in the blood supply and is evidence that ligation of the vessels does not effectively occlude all blood flow to the area involved. For example, at one or two days what appears to be a massive infarct actually consists of a mixture of normal and degenerating fibers showing varying degrees of pathologic changes. It also lends support to the view that collateral channels play a role in furnishing a limited supply of blood, which, however, is insufficient to maintain the normal structural and functional integrity for long and eventually leads to the death of most fibers.[13] It should be emphasized that quantitative chemical analyses of early myocardial infarcts should be accompanied by histologic controls, because the tissue is unevenly affected. The effects of occluding the veins could not be assessed in this study.

The histochemical demonstration of a loss of stainable glycogen does not necessarily indicate that irreversible changes have taken place. However, chemical examination of tissues considered to be permanently injured has revealed that irreversible changes first occur sometime between 20 and 45 minutes after temporary occlusion of the coronary vessels.¶ This is the time at which we have observed that glycogen is appreciably reduced in the ischemic area. Structural alterations and an increase in the water content of the injured myocardium also appear at this time. In frozen-dried tissues the presence of excess water is readily observed because of the obvious ice crystal artefacts limited to the affected area.

¶ References 2 and 14-16.

Quantitative loss of cardiac glycogen after coronary occlusion or anoxia has been established for different species.# In dogs vascular occlusion lasting from ½ to 24 hours results in a lowered glycogen content of the affected area.* With temporary occlusion the results on glycogen content have not been consistent.† The variations are probably due to a number of factors, including differences in the duration of occlusion, variations in the homogeneity of sampled areas, time allowed for recovery, and the adequacy of the collateral circulation.

Visual estimation, as employed in the histochemical detection of glycogen, is only grossly indicative of quantitative chemical changes; thus, disappearance of stainable glycogen does not necessarily indicate complete loss of glycogen. A parallel study of microscopic histochemistry with quantitative chemical analyses will be necessary to determine the threshold below which staining will be negative for a particular tissue. Whether or not the histochemical changes reported here are related to changes in the more labile trichloroacetic acid fraction of the total glycogen ‡ remains to be determined. Correlation between microscopic estimations and changes in chemical determinations of total glycogen has been reported for the hamster heart.[24]

The high content of glycogen in Purkinje fibers was discovered long ago by histologic methods, and the early literature has been well reviewed.§ Chemical analyses of the horse heart have shown that the bundle of His contains more glycogen than the cardiac fibers, but this is not true in the human. The ability of the Purkinje fibers to survive anoxia may be due in part to the fact that they contain large glycogen reserves and have a lower requirement for oxygen than myocardial fibers.[27]

Normal dog myocardium undergoing postmortem autolysis shows a depletion of stainable glycogen similar to that observed in

# References 17-19.
* References 2-5.
† References 2, 16, and 20.
‡ References 21-23.
§ References 25 and 26.

infarcts. After five hours at 27 C most of the stainable glycogen disappears from the cardiac fibers, but much remains in the Purkinje fibers. Mowry and Bangle [28] observed in human hearts a gradual general decrease in stainable glycogen depending upon the length of the postmortem interval. Quantitative determinations on autolyzing cat hearts have also shown a rapid decrease in glycogen.[29]

The exact nature of the PAS-positive substance or substances in the degenerating myocardial fibers is not known. French [30] observed a similar reaction in degenerating myocardial fibers of potassium-deficient rats. Prior treatment with malt diastase or saliva did not affect the stainability. The possibility that insoluble lipids may be involved cannot be excluded.|| According to McManus and Cason,[10] the acetylation technique should establish the carbohydrate nature of the PAS-positive material. Pearse, however, has expressed some doubt on the usefulness of this method.[32]

## SUMMARY

In an attempt to establish the histochemical changes that occur in early myocardial ischemia, a series of experimental myocardial infarcts of predictable location was produced in 24 dogs. The animals were killed from 20 minutes to 42 days after ligation of the coronary vessels. Tissues from the injured and normal myocardium were examined by various histochemical techniques.

The following observations were made with the periodic acid-Schiff (PAS) technique:

The cytologic distribution of glycogen and other PAS-positive material is described for normal dog myocardium prepared by freezing-drying and chemical fixation.

Within one hour after vascular occlusion a marked reduction in stainable glycogen occurs in the ischemic area. This change is best observed in frozen-dried tissue.

In addition, a peculiar degenerative change occurs in ischemic fibers within the first few hours following vascular occlusion. It is characterized by a positive PAS reaction within the fiber, which becomes progressively darker with time. This reaction is not due to glycogen. This change is best observed in chemically fixed tissue.

When healing by granulation has started, increased quantities of glycogen are observed in some myocardial fibers immediately surrounding the infarct.

During autolysis the sequence of events is similar to that occurring in ischemic injury.

Zigmund Zudyk, Dorothy Foss, and Mae Krivich provided technical assistance.

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# Exogenous Hemochromatosis

## A Report of Three Cases

WILLIAM A. MORNINGSTAR, M.D., Cleveland

The patient with fully developed hemochromatosis presents an enlarged liver, bronze pigmentation of the skin, diabetes mellitus, and a form of sexual hypoplasia which is characterized by impotence and female distribution of the body hair. The disease is relatively uncommon and was noted at postmortem examination only four times in 5000 autopsied cases at the Bellevue Hospital.[1] The incidence at the Veterans Administration Hospital, Cleveland, has been somewhat higher. There are 5 cases in a series of 945 autopsies. These are similar to the 311 cases collected in 1935 by Sheldon.[2] All the patients were men and their respective ages were 37, 46, 54, 57, and 59 years.

The exogenous form of hemochromatosis is rarer than the endogenous type. The first case of the exogenous type was reported by R. M. Kark in 1937, and by 1948 twelve other cases were reported. These cases occurred in anemic patients who received from 12 to 290 whole blood transfusions. None of these cases presented the fully developed syndrome of classical hemochromatosis. Schwartz and Blumenthal, when they reported five cases, noted this fact and predicted that a fully developed case would be encountered. The first case of our series fulfills all the criteria of fully developed hemochromatosis; two other cases are included. These are patients who had received 254, 154, and 135 blood transfusions, respectively.

### REPORT OF CASES

CASE 1.—C. H., a 59-year-old white man, had frequent admissions to the hospital between September, 1946, and August, 1949. In 1912, at the age of 23 years, he had a weeping generalized skin lesion, which was treated with x-ray irradiation. This condition recurred in 1915, 1917, 1935, and 1937, and he was given a total of four courses of x-ray therapy. In 1938 he was admitted to another hospital, where a diagnosis of a refractory anemia, one which did not respond to liver or nicotinic acid, was made. From this time, the patient received multiple whole blood transfusions: 254 in an 11-year period. There was no history of exposure to benzene or to any hemotoxic drug.

The earliest appearance of pigmentation of the skin was difficult to determine because of dry scaling and the secondary chronic dermatitis. The pigmentation of the skin was first positively noted in August, 1947. At this time, it was diffuse, light brown, and most marked on the exposed surfaces. The pigmentation became progressively darker.

In November, 1948, the patient developed a severe diarrhea with steatorrhea, which responded to pancreatin. A glucose tolerance test, performed on Dec. 9, revealed the following results: fasting blood sugar 92 mg. per 100 cc.; ½ hour, 156; 1 hour, 185; 2 hours, 148, and 3 hours, 81 mg. All urine sugar determinations were negative during this test, as were all such determinations since 1946. The terminal hospital admission lasted from August to December, 1949. At this time, repeated urinalyses showed 1 to 4 + glucosuria, and fasting blood sugar levels ranged from 170 to 357 mg. per 100 cc. The glucosuria and hyperglycemia were difficult to control.

The patient was a fairly well-developed, poorly nourished white man, who was chronically ill, weak, and lethargic on his terminal admission. His blood pressure was 98/52, and his pulse rate was 68 per minute. Both the liver and spleen were firm and palpable below the costal margin. His skin was dry, scaly, and dark brown.

Laboratory studies revealed: NPN 32.8 mg. per 100 cc.; total protein 6.2 gm. with an A/G ratio of 2.9 to 1; serum chloride 89.8 mEq.; sodium 137.8 mEq.; potassium 4.9 mEq.; cholesterol 203.1 mg. per 100 cc.; cholesterol esters 119.8 mg. per 100 cc., and icterus index 8. Blood cell counts varied from 2,700,000 to 3,100,000 red blood cells per cubic millimeter, and hemoglobin was 10 gm. per 100 cc. White blood cell counts ranged from 2600 to 6400 cells per cubic millimeter, and the differential cell counts were normal. Sternal marrow studies performed in 1946 and 1947 showed almost complete absence of early erythroid forms and normal proportions of granulocytic cells and megakaryocytes.

From the Department of Medicine and Surgery, Veterans Administration Hospital, and the Department of Pathology, Western Reserve University.

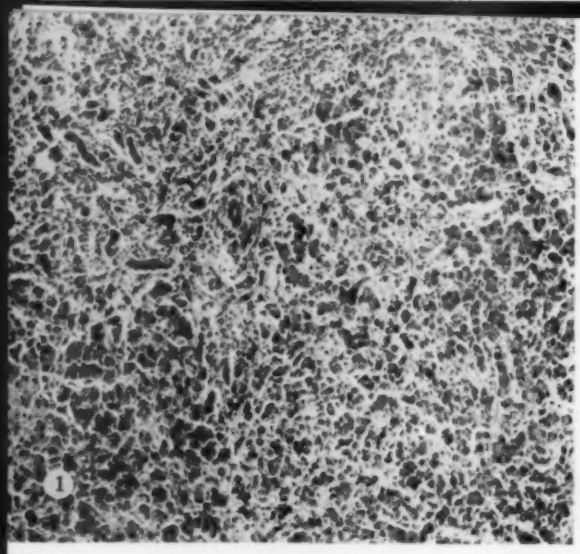

Fig. 1 (Case 1).—Liver, showing marked distortion of the architecture, some bile duct proliferation, marked fibrosis, and marked deposition of pigment; reduced ⅓ from mag. × 160.

The patient's course in the hospital was marked by slow but progressive weakness, low-grade fever, lethargy, and, at irregular intervals, mental confusion and occasional abdominal cramping pains, which were not localized. He died approximately four months after his last admission to this hospital.

On postmortem examination, there was no edema or abnormal collection of fluid. The heart weighed 350 gm. and had the normal structure. The myocardium was brown and moderately firm. The right and left lungs weighed 600 and 500 gm., respectively. There were bilateral patchy bronchopneumonia and moderate hyperemia. The spleen weighed 850 gm., and the cut surface was firm, finely granular, and dark reddish-brown, with sharp edges. The liver weighed 2600 gm., and the cut surface was finely nodular, dark brown, and firm. The pancreas weighed 110 gm., and the cut surface was flat, coarsely lobulated, and dark brown. The remaining viscera were not remarkable. Permission to examine the central nervous system was not given.

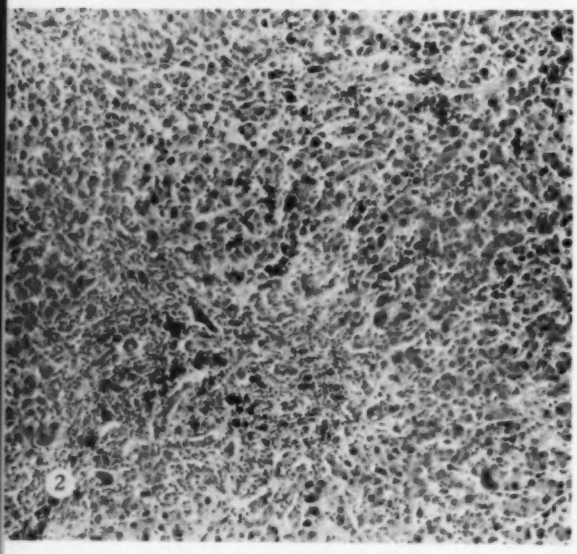

Fig. 2 (Case 3).—Liver, showing moderate distortion of the architecture, some bile duct proliferation, moderate perilobular and interlobular fibrosis, and marked deposition of pigment; reduced ⅓ from mag. × 160.

There was pigmentation on microscopic examination of the heart, lungs, liver, spleen, pancreas, bone marrow, lymph nodes, adrenals, kidneys, prostate gland, testes, and skin. The pigment was yellow to dark brown. The pigmentation was marked in the myocardial fibers and the interstitial macrophages of the heart. The pigment in the lungs was confined to the epithelial cells of the mucosal glands and the reticuloendothelial cells in the alveolar spaces and walls. The liver (Fig. 1) had pigment in the parenchymal cells, especially about the portal regions, as well as in the Kupffer cells and the epithelium of the bile ducts. The pigmentation of the pancreas was pronounced; it was present in the acinar cells, the islets of Langerhans, the epithelium of the pancreatic ducts, and the interstitial connective tissue (Fig. 4). The pigment in the spleen was contained in the reticuloendothelial cells and the fibrous connective tissue. The epithelial cells of the esophageal, gastric, and Brunner's glands also were pigmented. Pigment was heavily deposited in the zona glomerulosa of the

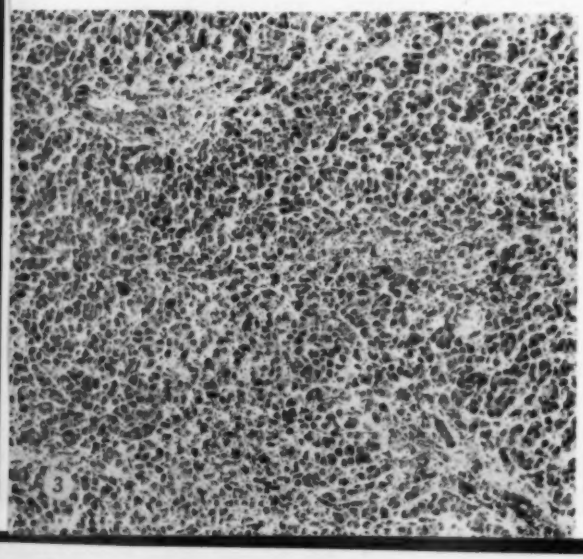

Fig. 3 (Case 2).—Liver, showing slight distortion of the architecture, slight periportal fibrosis, and marked deposition of pigment; reduced ⅓ from mag. × 160.

adrenal glands as well as in the outer portions of the zona fasciculata. The pigment was in the reticuloendothelial cells of the bone marrow and testes. The pigment stained intensely for iron throughout all the viscera.

Sections of the liver showed marked distortion of the lobules by fibrous connective tissue, which was primarily in the portal regions and extended into and through the adjacent lobules. There was also marked disruption of the normal pattern of the pancreas by interacinic and perilobular bands of fibrous connective tissue.

Sections of bone marrow revealed a cellular marrow composed predominantly of granulocytic cells, a normal number of megakaryocytes, and only a few erythroid cells. There was no fibrosis of the marrow.

There was marked replacement of the seminiferous tubular epithelium by Sertoli cells and there was aspermia. There was a marked increase of the fibrous connective tissue about the tubules and a moderate increase in the interstitial fibrous connective tissue (Fig. 5).

The brown pigment was present in the perivascular and periappendical regions of the skin and within the epithelial cells of the sweat glands.

In summary, this case is that of a 59-year-old white man with the four cardinal features of hemochromatosis: pigment cirrhosis of the liver, pigmentation of the skin, diabetes mellitus, and testicular atrophy.

Case 2.—The second case occurred in a 59-year-old white man, who had numerous admissions to the hospital during a four-and-one-half-year period beginning in September, 1945. A diagnosis of aplastic anemia was made, and the condition did not respond to liver, powdered stomach (Ventriculin), folic acid, ferrous sulfate, and 135 whole blood transfusions. Subsequently the patient developed generalized osteoporosis, with compression fractures of the third and eighth thoracic vertebrae. He also had hepatic and splenic enlargement. He developed anorexia, headaches, weakness, hemoptysis, and agranulocytosis. The red blood cell count was 720,000 cells per cubic millimeter and the white blood cell count was 2150 cells per cubic millimeter. The differential white blood cell count showed 80% lymphocytes. The patient had a normal platelet count, prothrombin, bleeding and clotting times, and glucose tolerance test. His skin gradually became darker, and his cough and dyspnea terminally grew progressively worse. He died June 15, 1949.

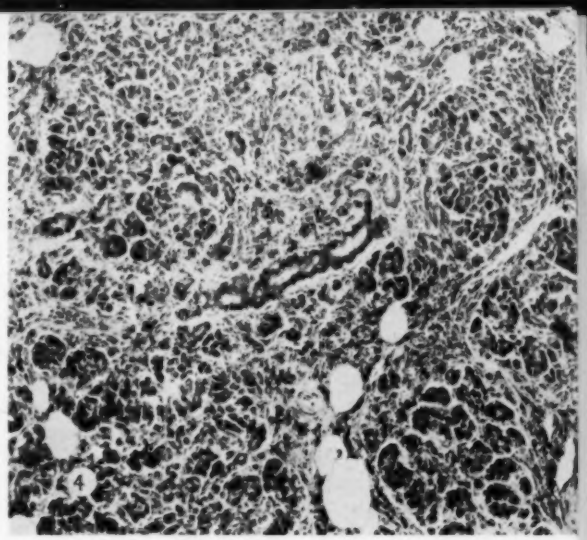

Fig. 4 (Case 1).—Pancreas, showing marked perilobular and interacinic fibrosis and marked pigment deposition with islet, acinar, and ductal cells; reduced ⅓ from mag. × 160.

The essential postmortem findings were pancytopenia with hypoplasia of the bone marrow; hemosiderosis of the liver (Fig. 3), spleen, pancreas, lymph nodes, lungs, kidneys, adrenals, and skin; osteoporosis of vertebrae and ribs; metastatic calcification of lungs and kidneys; bilateral chronic pyelonephritis, and atrophy of the testes.

This case is similar to Case 1 in the large number of transfusions, the aplastic anemia, the pigment cirrhosis, the pigmentation of the skin, and atrophy of the testes. However, there was no diabetes mellitus, no pigment within myocardial fibers, less pig-

Fig. 5 (Case 1).—Testis, showing aspermatogenesis with proliferation of Sertoli cells; reduced ⅓ from mag. × 160.

ment in the pancreas, and an acellular bone marrow.

CASE 3.—The third patient, J. H., a 60-year-old white man, had chronic monocytic leukemia and, during a three-year period, received 154 blood transfusions. He also had a chronic gastric ulcer, for which a gastroenterostomy was done in April, 1950. At this time, he had 80 transfusions and during operation a celiac lymph node was removed. In July, 1951, after 126 blood transfusions, a biopsy of skin from the right deltoid region was taken. Histologically, the lymph node showed only a few macrophages which contained brown iron-containing pigment. No iron-containing pigment could be demonstrated in the skin biopsy.

Postmortem examination revealed leukemic infiltration of the splenic pulp, lymph nodes, bone marrow, and liver. There was marked aplasia of the bone marrow. There was a marked deposition of hemosiderin in the myocardium, lungs, bone marrow, spleen, lymph nodes, liver, and pancreas. There was slight to moderate deposition of hemosiderin in the kidneys, testes, adrenals, and submucosal glands of the duodenum and in the skin. There was slight perilobular and periportal fibrosis of the liver (Fig. 2) and moderate interlobular and interacinic fibrosis of the pancreas.

The third case exhibited less pigment deposition in the viscera than did the first case and a greater quantity than is present in the second case. There was marked pigment deposition in the myocardium. Diabetes mellitus is the only feature of a fully developed case of hemochromatosis which is lacking.

## COMMENT

A severe case of exogenous hemochromatosis is indistinguishable from hemochromatosis of unknown etiology. The three cases presented here suggest that the deposition of hemosiderin occurs in an orderly fashion. The histories concur with Finch and associates [3] that over 200 transfusions are usually necessary to produce a picture of fully developed hemochromatosis. Marked pigment deposition was found in the liver, spleen, and pancreas of all three cases reported. The two cases which received 154 and 254 transfusions, respectively, showed marked hemosiderin deposition in the myocardium, sweat glands, periappendicular and perivascular regions of the skin, and within the submucosal glands of the duodenum. No pigment could be demonstrated in these locations in the case which received 135 transfusions. The progressive deposition of hemosiderin is also suggested by the last case, in which a celiac lymph node biopsy after 80 transfusions showed scant pigment and a skin biopsy after 126 transfusions revealed no pigment, whereas in comparable sites at autopsy after 154 transfusions, large amounts of hemosiderin were found in both locations.

In these three cases (Figs. 1, 2, and 3) the increasing amount of perilobular fibrosis in the liver parallels the number of transfusions. The patient who received only 135 transfusions exhibited slight periportal and perilobular fibrosis. The patient who received 154 transfusions had only a moderate amount of fibrous connective tissue in these locations, whereas the patient who had received 254 transfusions had marked fibrosis with disruption of the normal architecture, bile duct proliferation, and disappearance of the central veins of the liver lobules. The changes in the pancreas of the three patients also revealed a correlation between the number of blood transfusions and the amount of pigment, degree of fibrosis, and distortion of architecture.

## CONCLUSION

Three cases of exogenous hemochromatosis are presented in which many blood transfusions were given. The degree of pigmentation as well as the degree of fibrosis appeared to be a direct function of the number of transfusions. A patient who received 254 blood transfusions exhibited all the features of idiopathic hemochromatosis.

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# Organoid Neuroectodermal Neoplasms of Skin

## A Histopathologic Study of a Morphologic Variant of Juvenile Melanoma

ROBERT JOSEPH PEACE, M.D., New Orleans

This report is based on the histopathologic study of two neoplasms of skin that, because of their unusual histopathologic characteristics, could not readily be classified according to conventional diagnostic schemes. Its object is to record the morphology of these neoplasms, giving particular emphasis to their distinctive cytologic composition and to their complex structural organization. In its course, I will express my concept of the nature of the lesions by applying to them the term "organoid neuroectodermal neoplasms of skin" and will attempt to relate them to juvenile melanomas.

The two lesions were strikingly similar in gross and microscopic appearance, and in both a pseudoalveolar "endocrine" pattern was readily apparent upon scanning histologic sections with the lower-power objective lenses. Both were thought by most consultants to represent a form of benign melanotic neoplasm, and, while many could recall examples of similar lesions from past experience, none could offer a satisfactory diagnostic term. The remarkable, almost coincident, occurrence of two such neoplasms in the routine material of one laboratory, coupled with the interesting problem of their diagnostic classification and relation to melanotic and other neurogenic neoplasms, prompted the study which is the basis of this report.

### REPORT OF TWO CASES

CASE 1 (B. E. G., USNH CLNC Reg. No. 61855).—A 22-year-old Caucasian male Marine corporal complained of a tumor of the skin of the instep of the left foot. This tumor had been present for six years, having appeared suddenly without antecedent trauma, and had grown rapidly over a period of months until it had attained a constant size. At first it was dark red, firm, and had on one occasion bled excessively after trauma, but eventually it became lighter in color, flabby, and ceased to cause the man concern. Upon entry into military life he found that prolonged standing or marching made the tumor swollen and painful.

At a point over the proximal head of the first metatarsal bone was a dusky, hemispheric, soft tumor which measured 1.8 by 2 cm. in greatest dimensions. It was poorly circumscribed and, when exposed at surgical operation, was found to extend to the plantar fascia. It was excised with a portion of uninvolved tissue and the defect filled with a split-thickness skin graft. The surfaces made by cutting the tumor were light yellow-orange, dry, and flat. It was limited to the dermis, graded imperceptibly into the contiguous fibrous tissue, and pushed upward, carrying over it the intact epidermis.

Histologic sections (S52-1690) showed a poorly circumscribed dermal neoplasm composed of large polyhedral, oval, or fusiform cells, arranged in complex pseudoalveolar or tubular structures separated by dense fibrous lamina or acellular hyaline matrix (Fig. 1). The fibrous stroma was loosely knit in the central portions of the tumor, heavily infiltrated with lymphocytes and plasmacytes, and accompanied throughout by a prominent capillary vascular component. Although the over-all microscopic appearance was somewhat suggestive of that of a malignant melanoma, there were no pigment granules and there were no cell nests at the junction of the epithelium and the subepithelial fibrous tissues. Mitotic figures were absent, and, despite the striking degree of cellular pleomorphism, there was no actual cytologic evidence of malignant neoplasia. In consultation, the Armed Forces Institute of Pathology reported the lesion (AFIP acc. 563840) to be "a cellular nevus, for want of a better term." It was also suggested that this lesion might be an atypical sweat gland carcinoma. When last seen, 12 months after excision of the tumor, the patient was well and without evidence of recurrence or metastasis.

CASE 2 (R. T. P., USNH CLNC Reg. No. 62862).—A 22-year-old Caucasian male Marine cor-

From the Laboratory Service, United States Naval Hospital, Camp Lejeune, N. C.

Lieutenant, Medical Corps, United States Naval Reserve; present address: 2209 Calhoun St.

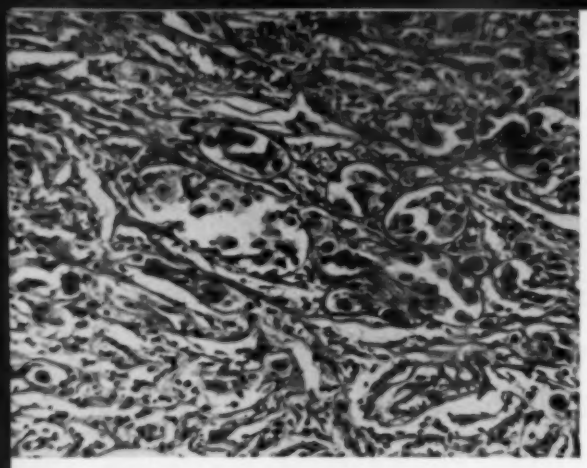


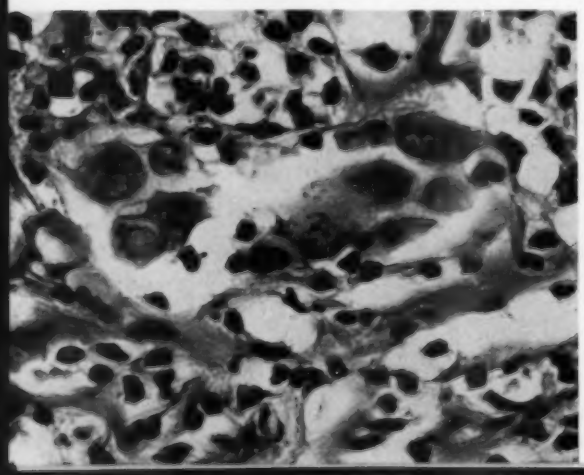
Fig. 1 (Case 1).—Typical microscopic field of neoplasm to demonstrate large neuroectodermal ganglionic cells arranged in tubular or pseudoalveolar structures. Note the large nuclei and prominent nucleoli. Reduced ¼ from mag. × 100.

poral complained of a tumor of the skin of the anteromedial surface of the left leg. This tumor had been present for four months, having appeared, without antecedent trauma, as a small red pimple which had increased in size slowly but progressively.

At a level 10 cm. below the knee was a dusky pink, hemispheric, soft and rubbery tumor which measured 1 by 1.5 cm. in greatest dimensions. It was excised with a portion of uninvolved tissue and the defect filled with a full-thickness skin graft. The surfaces made by cutting the tumor were light orange-pink, dry, and retracted. It was limited to the dermis, poorly demarcated from the adjacent fibrous tissues, and in extending upward carried over it the intact epidermis.

Histologic sections (S53-188) showed a neoplasm composed of polyhedral, fusiform, or comma-shaped cells arranged in cords, tubules, or pseudoalveolar structures disposed in a coarse framework of dense, heavily collagenized subepithelial fibrous tissue (Figs. 3 and 4). The over-all microscopic appearance suggested in many ways an intradermal nevus. However, there was no demonstrable melanin pigment, there were no junctional cell nests, and the overlying epithelium was thin and without skin-appendage structures. In consultation, the Armed Forces Institute of Pathology reported the lesion (AFIP acc. 586447) to be "a form of neuronevus," although the possibility of its being a reticulohistiocytoma or myoblastoma was mentioned. When last seen, approximately 10 months after excision of the tumor, the patient was well and without evidence of recurrence or metastasis.

Fig. 2 (Case 1).—Detail of characteristic neoplastic cells and structural organization. The cytoplasm is abundant, arranged in pseudosyncytial strands, and is granular. One large cell near the left end of the space contains an intracytoplasmic inclusion body. Reduced ¼ from mag. × 400.



## HISTOPATHOLOGIC OBSERVATIONS

The finer histopathologic details of these neoplasms are striking and of sufficient interest to warrant further consideration as to their possible nature and origin. In both, the characteristic neoplastic cells had large, sometimes irregularly lobulated or folded nuclei that were often eccentric and varied in size from one cell to another. There were many cells with multiple nuclei, often as many as five or seven in a single cell. The nuclear substance was loosely knit, although frequently there was peripheral clumping and irregular knotting around the nuclear margins. All of the cells had prominent, usually brilliantly acidophil, nucleoli. A remarkable feature, particularly in many of the larger multinucleated cells of the first lesion, was the presence of peculiar intranuclear and intracytoplasmic inclusions (Fig. 2). These inclusions were somewhat refractile, generally ovoid, and did not stain specifically. They sometimes appeared to be hollow spherules.

The cytoplasm was generally abundant, particularly in the cells of the first lesion, finely granular or fibrillar, and sometimes fenestrated. Occasional cells had a foamy appearance which suggested tiny, finely dispersed vacuoles, but fat could not be demonstrated in the cells of either tumor. The cytoplasm of some cells had a "ground-glass" appearance and assumed a reddish-brown hue with trichrome stains. There were occasional accumulations of coarse intracytoplasmic granules, which were dark blue in sections stained with hematoxylin and eosin and bright red in sections stained with a trichrome mixture. The cell borders were often fuzzy or serrated, and there was a

tendency to the stringing-out of cytoplasmic processes to form pseudosyncytial unions. All these cells possessed the morphologic characteristics of primitive neuroectodermal cells and frequently bore an extraordinary resemblance to adult ganglion cells.

The neoplastic cells were arranged along sinusoids or channels incompletely lined with flattened cells and sometimes having only a thin condensed membranous lining. The littoral cells were elongated and had oval pyknotic nuclei, without distinguishable nucleoli, and metachromatic cytoplasm. They did not possess the characteristics of endothelial cells but instead bore a strong resemblance to cells of the lemmoblastic capsule around ganglion cells. Frequently the large neoplastic cells lay along the channels in close contact with the littoral cells and membrane, often appearing to fuse with the lining. The sinusoids or pseudoalveoli were often separated by abundant acellular or hyaline matrix, which blended into normal collagenous tissue at the periphery of the neoplasm. Neural elements in the form of Meissner-corpuscle-like structures were present in some portions of the lesion, particularly in the second, and were often intimately related to thickened small arterioles.

In summary, it may be said that these were neoplasms composed of distinctive neuroectodermal "ganglionic" cells, which were remarkable by reason of a high degree of organization into complex pseudoalveolar "organoid" structures. To express my concept of their structure and origin, I will refer to them as "organoid neuroectodermal neoplasms of skin."

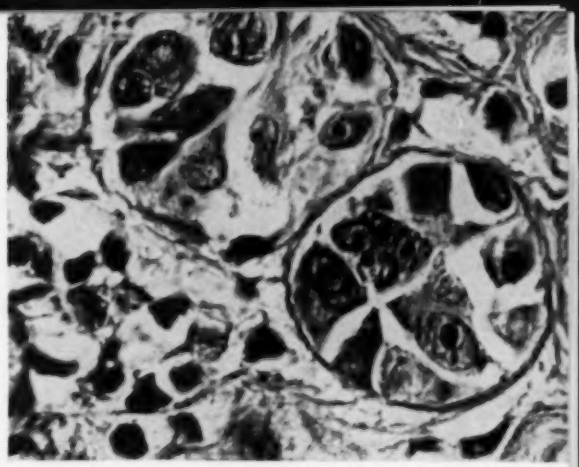
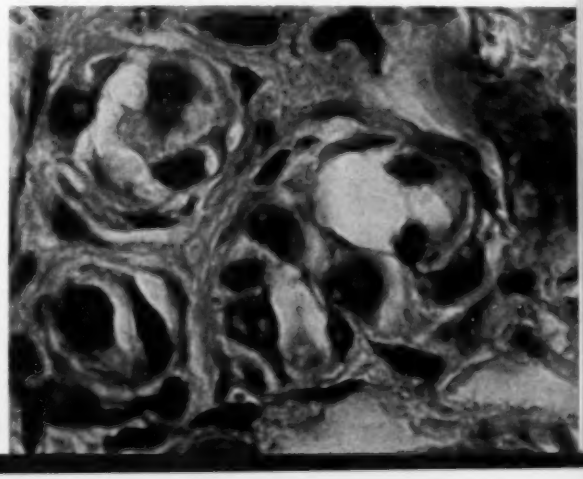


Fig 3 (Case 2).—Detail from the second neoplasm to demonstrate the alveolar arrangement and the characteristic cytologic detail of the neoplastic cells. Reduced ¼ from mag. × 450.

## COMMENT

Although it was impossible to assign these lesions a definite diagnostic classification, it was possible to relate them on grounds of morphologic similarity to the general group of benign melanotic neoplasms. Despite one sharp point of dissimilarity, in that the organoid neuroectodermal neoplasms of this report had no junctional nevus cell component, and despite the fact that both patients were past puberty, several common structural peculiarities make it possible to relate them to the so-called "juvenile" melanomas described by Allen and Spitz.[1] McWhorter and Woolner [2] have recently reported a case of a juvenile melanoma without junctional component, and juvenile melanomas have been observed as late as the fourth decade of life. Both the lesions of this report and the juvenile melanomas are characterized by the presence of large cells with abundant, faintly oxyphilic or ground-glass cytoplasm, large multinucleated cells sometimes reminiscent of Touton giant cells, stromal vascularity, and lymphocytic infiltration. Juvenile melanomas tend to form sharply segregated, compact cell nests; in this connection it is interesting that Figures 7 and 8, illustrating Spitz's original paper on this subject,[3] depict a structural pattern similar to that of the two lesions reported here and, although not noted in the captions, illustrate beautifully the organoid arrangement which I have emphasized. Because of the short follow-up time,

Fig. 4 (Case 2).—Detail from second neoplasm to illustrate cytologic detail suggesting that of peripheral neural receptor organs. Reduced ¼ from × 450.



no parallel can be drawn between the biologic behavior of these organoid neuroectodermal neoplasms and that of juvenile melanoma.

The organoid or "endocrine" arrangement of the neuroectodermal cells suggests a possible relationship between these neoplasms and the alveolar soft-parts sarcomas described by Christopherson and others [4] and that similar group of lesions described by Smetana and Scott as "nonchromaffin paragangliomas." [5] The intimate relationship of the neoplastic cells to sinusoids was strongly reminiscent of the structure of nonchromaffin paraganglionic tissue, and, incidentally, the anatomic location of the two lesions of this report fits in with the concept of peripheral disposition of such tissue which has been expressed by Smetana and Scott.

Because of a superficial resemblance of some of the large multinucleated cells of the tumors of this report to some forms of histiocytes, the possibility is raised that they might well be included in that heterogeneous group of lesions reported in the dermatologic literature under the title "reticulohistiocytoma." * It would seem that reticulohistiocytoma is a separate disease entity, the nature of which is that of a systemic reactive disease possibly related to Letterer-Siwe disease and characterized by a syndrome of polyarthritis associated with multiple nodular skin tumors composed of histiocytes. Histologically, reticulohistiocytoma is differentiated by the lack of structural organization, as in the organoid neoplasms, and by the presence of an associated general mesenchymal cellular response.

* References 6 and 7.

SUMMARY

Two unusual neuroectodermal neoplasms of skin are reported, with a detailed description of histopathologic findings. Although they could not be definitely classified, their morphology was such that it was possible to relate them to the general category of melanotic neoplasms, and it was felt that they represented morphologic variants of so-called juvenile melanoma, despite the fact that there were no junctional elements.

Cmdr. G. E. Meador (MC), U. S. N., Chief of the Laboratory Service, U. S. Naval Hospital, Camp Lejeune, N. C., at the time, gave advice and encouragement in preparation of this report. Members of the Department of Medical Illustration of the Louisiana State University School of Medicine assisted in preparing the photomicrographs.

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# Fatal Rupture of Gastric Aneurysm

**Case Report with Review of the Literature**

MAX MILLARD, M.B., M.R.C.P.I., D.C.P., Miami, Fla.

Present-day indications for surgery in cases of hematemesis generally allow 24 hours of expectant therapy if the bleeding is continuous or repeated. The purpose of this paper is to report a case of fatal hemorrhage from an aneurysm of one of the smaller submucosal branches of the gastric arteries. This condition has been rarely described and is almost certainly fatal without surgical intervention.

## GASTRIC HEMORRHAGE

In recent years it has become apparent that the emergency diagnosis of a perforated peptic ulcer or of ulceration into a gastric vessel does not explain all causes of hematemesis. Rarer causes have been reported, such as severe hematemesis in which examination of the removed stomach shows no macroscopic lesions; a single case of schistosomiasis in the stomach (Willard *); an erosive type of atrophic gastritis with hemorrhage from venocapillary ectasia, reported by Rider, Klotz, and Kirsner.[1] In an extensive review of the subject of vascular malformations and vascular tumors of the gastrointestinal tract, Gentry, Dockerty, and Clagett,[2] in 1949, discussed practically all possible causes of gastric hemorrhage, reviewed the literature, and yet made no mention of gastric aneurysm.

There are reports of ruptures of the main gastric arteries, sometimes of the dissecting type of aneurysm, with massive intraperitoneal hemorrhage (Shumway and Peyton[3]); multiple aneurysms of splenic, gastric, renal, and superior mesenteric arteries (Levy[4]), in which the left gastric vessel was involved in an arteriosclerotic aneurysm near the celiac axis; the case of Melendez and Miranda,[5] in which a left gastric artery aneurysm perforated into the stomach; the case of Taylor,[6] who reported a ruptured anastomotic aneurysm at the junction of the right and left gastric arteries. This aneurysm ruptured into the peritoneal cavity, not the stomach.

A further cause of severe gastric hemorrhage is hemangioma. Such a case has been reported by Kraus,[7] and the review by Gentry and co-workers[2] lists many similar conditions. Hemangiomas are commoner in the intestine, and the report of a case of cavernous hemangioma of the jejunum, by Butler, Tillisch, and Priestley,[8] draws attention to Kaijser's[9] postulates as to their origin. Kaijser considered that they might arise from (1) congenital rests, (2) congenital mesodermal defects in the intestinal wall with dilatation, (3) local irritation with dilatation and new vessel formation, and (4) congenital arteriovenous anastomosis.

## ANEURYSMS OF JEJUNUM

In reviewing the literature relative to this case, two reports of aneurysm in the jejunum were found: Rathmell, Horwell, and Greeley[10] reported a case of a 29-year-old white woman in good health until the onset of sudden massive intestinal hemorrhage. The source could not be found at laparotomy. Only at autopsy was a 0.5 cm. lesion found in the mesenteric border of the second part of the jejunum. Microscopic sections showed a thick-walled vessel with a perforation at a point where the media was thinned. No inflammatory reaction or arteriosclerosis was seen.

Levine and Valk[11] reported an aneurysm with rupture of a submucosal artery in the

Department of Pathology, University of Miami School of Medicine, and Department of Pathologic Anatomy, Jackson Memorial Hospital.

* Willard, to be published.

jejunum in an 18-year-old Negro woman. This patient had had sudden copious melena for three days, as her first symptom. Conservative treatment followed by laparotomy two weeks later showed, about 20 to 30 cm. from the origin of the jejunum, a small bluish papule, the size of a hazelnut. This was excised and most of its size was seen to be due to blood clot. On removing the latter, a small elevated (unmeasured) cordlike mass was left. Microscopy showed, in the submucosa, a large thick-walled vessel, 4 mm. in diameter, which elevated and compressed the mucosa and muscularis mucosae and was filled with thrombus. It had ruptured. Two separate coats were demonstrated, poorly defined. There was an outer musculoelastic coat and an inner proliferated fibroblastic cellular tissue. No elastica could be demonstrated between them. At one point the differentiation between the coats was fairly distinct. This was at the region of two small evaginations or branches from the main vessel. Here, the musculoelastic layer was thick and well preserved and continued into the branches. Elsewhere, the coat was edematous and poorly preserved and showed numerous violaceous foci of degeneration, some of them with small hemorrhages. There were similar foci of degeneration with hemorrhage in the fibroblastic intimal layer. The outer portion of the musculoelastic layer and the immediately surrounding fibrous tissue (the latter corresponding to the adventitia) was sprinkled with a few round cells and polymorphonuclear leucocytes. These were most numerous, but by no means diffuse or heavy, near the site of rupture and near the compressed muscularis mucosae. The endothelial lining was almost completely destroyed. The thrombus showed some peripheral organization and canalization. The lateral edges of the projecting thrombus showed slight epithelization from the adjoining mucosa. Other vessels and the rest of the intestinal wall were normal. Because of the disorganization of the vessel wall and the absence of a definite elastica interna, a varix had to be ruled out. Special stains showed definite concentration of musculoelastic tissue in the outer half of the wall and, also, in the "branches." Secondary degenerative changes in an aneurysmal wall may lead, the author thought, to destruction or disappearance of the media.

In an article on congenital arteriovenous and cirsoid aneurysms, Adams[12] mentions the possibility of congenital aneurysms in the stomach but does not cite a case.

## REVIEW OF LITERATURE

The first recorded cases of gastric aneurysm with hemorrhage are those of Gallard[13] (1884).

The first patient was a 20-year-old male who complained of severe epigastric pain and suddenly vomited 3 liters of blood. Hematemesis continued for two days, after which death ensued. Autopsy showed a small ruptured aneurysm in the posterior wall of the stomach, near the lesser curve of the cardiac region. It was in the center of a small erosion, and microscopic sections showed hardly any arteriosclerosis.

The second patient was a 51-year-old person, sex unstated, in whom, again, there was a sudden hematemesis and melena. The postmortem examination revealed, in the posterior wall of the stomach, 7 cm. below the lesser curve and 2 cm. from the cardia, a "grit"-sized erosion, which showed a ruptured miliary aneurysm in the center.

A third case was not analyzed in detail.

Gallard further mentioned that Lionville discussed two cases and Riegal described one similar case. They are also cited in Hauser's article in Henke and Lubarsch.[14]

In 1892 came the next report, which was from Sachs.[15] This patient was a 79-year-old man with a history of hematemesis. At autopsy the stomach was filled with coagulated masses of blood, after removal of which a smooth nonulcerated mucosa was seen. The origin of the bleeding was at first obscure. Finally, at about the middle of the lesser curve there was found a fine coagulum, under which an opening in a 5 mm. protrusion, rising 1½ mm. above the surface, was found. The coagulum lay over a small submucosal artery at a point where it described an arch,

the convexity of which projected into the mucosa. The artery was sharply kinked, producing a spur protruding into the lumen of the vessel. At its concavity there was typical normal media; the intima was thickened, narrowing the lumen. This thickening abutted against the convexity, where all evidence of media was lost, and the whole wall consisted of a structureless mass, its thickness decreasing as it came farther away from the normal part of the wall. The rupture was plugged with fibrin and red blood cells. The gastric mucosa was closely attached to the artery, was thinned over it, and, finally, ulcerated. The surrounding mucosa was heavily infiltrated by round cells. Sachs considered himself lucky that the fibrin led him to discover the aneurysm and published his case with the comment that perhaps many such cases had occurred.

In 1904 Hirschfeld [16] reported the case of a 38-year-old man who, in the last year of his life had had 12 heavy hematemeses, 10 of which caused hospitalization. He died during the 13th hematemesis, after four days. At autopsy, on the posterior wall of the stomach there was a pinpoint protrusion of yellowish-white color. Serial sections showed an approximately 3 mm. mucosal defect, the base of which was formed by the submucosa. In the center was an aneurysm which had ruptured and was occluded by partly organized thrombus. The surrounding submucosa was infiltrated by round cells. As the patient was a hemophiliac and originated from a bleeder family, Hirschfeld did not say whether the patient would have died without his hemorrhagic diathesis. He considered that excessive alcohol was mainly responsible for the production of the aneurysm, with hemophilia as a secondary cause.

In 1931 Hess [17] mentioned a gastric dissecting aneurysm with embolism. Loeper and Lemaire,[18] in 1932, reported a case of an aneurysm of Rasmussen in a callous ulcer in the stomach. This, of course, is in a group entirely apart from that which we are discussing.

Hoelzer,[19] in 1936, reported a further fatal hemorrhage from a miliary aneurysm of an artery in the gastric mucosa. This patient was 84 years old and had had 10 days of hematemesis, dying on the 14th day. The clinical diagnosis was carcinoma of the stomach. At autopsy the entire gastrointestinal tract was full of dark red blood and all organs were greatly anemic. Marked arteriosclerosis was noted. Apart from an old ulcer scar in the duodenum, the mucosa of the whole gastrointestinal tract was smooth. On the anterior wall of the stomach, 4 cm. from the cardia and 5 cm. from the lesser curve, was a defect 4 mm. in diameter and 0.5 mm. deep. In its center was a drop of bright red blood. The surrounding vessels were readily visible, tortuous, and indurated. A gross diagnosis of varix or aneurysm was made, and the microscopy showed that the vessel involved was an artery. The surrounding mucosa and the arterial coats were well preserved. There was a bulge of the mucosa, at which point intimal thickening in the underlying artery was noted. Above the vessel the mucosa was chronically inflamed. Deeper slides showed an increasing bulging of the intima and thinning of the media, with medial necrosis and degeneration of muscle nuclei (karyorrhexis). The intimal proliferation was noted to be present only in the bulging part of the artery. In the rest of the circumference there was no such change. In the thick intima hardly any elastica was present. In the center of the mucosal defect the submucosa was also absent, and the artery was eroded to a large extent and partly occluded by a mixed thrombus. The other gastric arteries showed no marked change except some mild arteriosclerotic intimal thickening. In his discussion Hoelzer considers two possibilities: 1. A preexisting hemorrhagic erosion. There are several reasons for nutritional disturbance in the mucosa due to the changes in the gastric arteries which could delay regression and healing of the erosion. After some time, a local digestion of the submucosa could have taken place, and even the vessel itself could have been digested. In such a case it would be necessary to postulate, as a consequence of vascular damage, a compensatory intimal thickening. Then erosion would

occur at the dome of the bulging part of the vessel. 2. The second possibility is to consider the aneurysm as arteriosclerotic. It is perfectly feasible that the pressure of such an aneurysm would impair mucosal and submucosal nutrition, leading to digestion, including that of the aneurysm. A callous ulcer, as a cause of the erosion, could easily be excluded by microscopic studies.

Next, in the European literature, comes an important study from Italy by Bianchedi.[20] In a review entitled "Anatomical Contributions to the Study of Gastric Aneurysms," he reviews 11 cases of atheromatous widening, some with rupture. He lists several references already cited in this paper and also mentions Scheidegger,† who reported what he considered to be a congenital aneurysm in the gastric mucosa. There was no lesion in the walls and other dilated vessels were seen in the gastric wall with, in addition, multiple varices throughout the gastrointestinal tract. In the group of congenital aneurysms or telangiectases, Bianchedi refers to a case of Delore, Comte, and Labry,‡ who observed a vascular, pulsating stomach with noteworthy proliferation of vessels which were tortuous and ectatic. There was a reference, also, to a case of Hanser,§ with a traumatic aneurysm due to a swallowed needle. He notes, too, that Luys ‖ and Hirschfeld ¶ pointed out an analogy with the aneurysms found in the gastric mucosa of alcoholics. He mentions that Bitot and Papin # saw ulcerations near tortuous ectatic vessels. There is also reference to the role of periarteritis nodosa in the causation of aneurysm, and he remarks that, although embolic aneurysms are theoretically possible, none have been reported.

Bianchedi's own case was that of a 40-year-old man. The autopsy revealed that the mucosa in the fundus and its neighborhood was markedly rugated. Six centimeters from the pylorus, on the posterior wall, was a large pinhead-size protrusion covered by thrombus. The mucosa around it appeared atrophic and slightly eroded. Histological sections revealed that this was an arterial branch with marked changes in the wall, consisting of increased elastica and connective tissue in the intima, with disorganization of the internal elastic lamina and hazy subendothelial tissue. The media was well preserved. The adventitia at the point of the thrombus was reduced to a few fibers and here the mucosa was absent. To either side, it was nearly atrophic and was partly replaced by poorly cellular connective tissue, infiltrated with lymphocytes and polymorphonuclear leucocytes. Other vessels showed fibrillation of the internal elastic lamina, especially near the mucosa. At the point of greatest projection of the vessel, the media was poor in nuclei, thin, and was amorphous, pink, and necrotic. Here, no external elastic lamina or adventitia could be seen. The neighboring mucosa was hypertrophied, almost villous, and the glands were increased in number. There was a submucosal connective tissue infiltrated with lymphocytes. No other lesions were noted. The presence of elastic connective tissue at the point of aneurysm indicates slow development (compare Jores compensatory endarteritis). The author thinks that aneurysms probably occur only when there is medial and intimal damage, which, in the latter case, is in the elastica. However, he points out that Benda states that the connective tissue must be simultaneously destroyed.

The next reference appears to be in 1946, when Heuer,[26] discussing the surgical aspects of hemorrhage from peptic ulcers, cites a personal case of massive hemorrhage with an apparently normal stomach. At autopsy, until fluid was injected into the gastric vessels, demonstrating a ruptured aneurysm below the intact mucosa, no cause for the hemorrhage could be ascertained. The microscopic report is not cited.

In the same year a case of cirsoid aneurysm of the left gastric artery, with secondary peptic erosion, was presented at the Massa-

† Scheidegger,[21] cited by Bianchedi.[20]

‡ Delore, Comte, and Labry,[22] cited by Bianchedi.[20]

§ Hanser,[23] cited by Bianchedi.[20]

‖ Luys,[24] cited by Bianchedi.[20]

¶ Hirschfeld,[16] cited by Bianchedi.[20]

# Bitot and Papin,[25] cited by Bianchedi.[20]

chusetts General Hospital. In the ensuing discussion Mallory * stated that the case was unique to him. His microscopic preparation showed that a sclerotic, tortuous artery rose up to the mucosa, and its pressure was assumed to have caused ulceration from which hemorrhage resulted. He could not say that the aneurysm was congenital or acquired, neither did he know how this could be ascertained. Therefore, he called the aneurysm cirsoid.

Donaldson and Hamlin [28] reported, in 1950, three cases of massive hematemesis, resulting from a gastric artery aneurysm. The patients were 55, 59, and 64 years old. Patient No. 1 had a successful gastrectomy. The stomach showed a ruptured cirsoid aneurysm of a branch of the left gastric artery. It was 4 mm. in diameter and raised 1 mm. from the surrounding mucosa. A blood clot overlay a defect in the artery, measuring 2.5 mm. in diameter. The aneurysm took a tortuous course, without diminution in caliber, and came to lie in the basal portion of the submucosa, where it was disrupted and then turned downward again. It was seen to have yellowish atheroma, as did the other gastric vessels. Microscopy showed absence of the mucosa which was replaced by a recent laminated clot, covering a large defect in the artery, which was lying in the base of the mucosa. The vessel was 3 mm. in diameter and unusually large. It showed arteriosclerosis with intimal thickening, and surrounding it was acute inflammation. Subtotal gastrectomy was performed on Patient No. 2 and showed hypertrophic gastritis, which was thought to be the source of the bleeding. The subserosal vessels were noted to be markedly arteriosclerotic and tortuous, and a duodenal ulcer scar was also removed. Postoperatively, hemorrhage continued and the rest of the stomach was removed. However, the patient died of a postoperative complication. The microscopic examination of the first specimen of the stomach showed chronic gastritis, whereas, in the second, a cirsoid aneurysm of a branch of the left gastro-

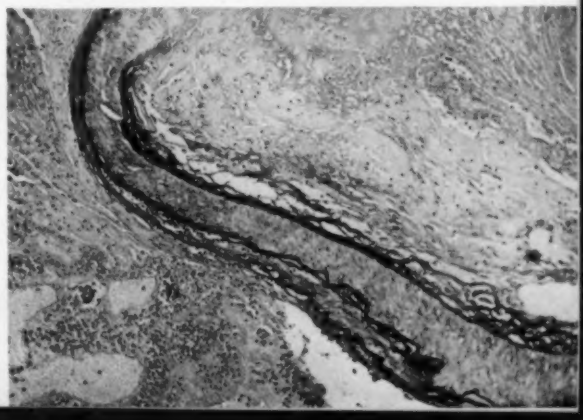
* Mallory, T., in discussion on Case No. 32402, Massachusetts General Hospital.[27]

Fig. 1.—Gastric aneurysm capped by blood clot; formalin-fixed.

Fig. 2.—Aneurysm now folded. Note disrupted ends merging with blood clot. The intima shows irregular thickening. Reduced ½ from mag. × 21.

epiploic artery was found to have ruptured and measured 2 by 2 by 2 mm. The vessel was dilated and sclerotic, as were the others in the stomach. Patient No. 3 died of his hemorrhage. Autopsy revealed that 5 cm. distal to the cardioesophageal junction on the greater curve was a slightly raised area of

Fig. 3.—One end of ruptured aneurysm. Reduplication of internal elastic lamina, medial degeneration, only slight intimal thickening. Reduced ½ from mag. × 122.



mucosa, 1 cm. in diameter, in the center of which was a 4 mm. shallow depression filled by thrombus. A tortuous artery ran beneath this and showed aneurysmal dilatation up to 3 mm. Microscopy does not mention the structure of the vessel.

Thus, this review of the literature has collected 15 undoubted cases of gastric aneurysms plus 1 incompletely proved case of Gallard. In addition, there is the collection of Bianchedi of 11 cases already mentioned, not all being congenital or arteriosclerotic.

## REPORT OF CASE

We now come to the case under present consideration, that of a 46-year-old white man, who one afternoon suddenly vomited a large amount of blood, about 2 qt. (2000 cc.), without clots. He was immediately sent to the hospital where, on admission, he was not then vomiting but had a copious melena stool, which was soft and black. The patient denied any similar previous illness. The patient was observed for four days, during which 21 pt. (10,500 cc.) of blood was given, in addition to large amounts of invert sugar (Travert) 10% in water, for repeated hematemesis. He was taken to the operating room and died with a large vomitus of blood before the induction of the anesthetic could be made.

*Autopsy.*—Because of difficulty in obtaining consent, the autopsy could not be commenced until 52 hours after death. Bloody fluid was running from the mouth and nose and was found to be extending down into the smallest branches of the bronchial tree. There was no visible abnormality of the coronary vessels or of the aorta. No abnormality was noted in the peritoneal surfaces. The organs were noted to be rather pale. The esophagus showed no abnormality and no varices. The stomach was distended with 3 to 4 pt. (1500 to 2000 cc.) of bloody fluid. Its mucosa was smooth and the wall thin. Midway between the lesser and greater curves on the posterior wall of the stomach was a projecting brownish nodule, 2 mm. in diameter, rising 1 mm. above the surface. As the surface was washed clean, blood repeatedly oozed from it. On holding the stomach up to the light, this projection was seen to be from a large artery running through the wall. Unfortunately, photography was not possible at this time and fixation had to be carried out. The rest of the intestine was filled with blood but showed no abnormality. The illustration (Fig. 1) shows the projecting aneurysm and surrounding, apparently normal mucosa. The fixation precludes demonstration of the large vessel running beneath the mucosa.

*Microscopy.*—The sections of the stomach (Fig. 2) show remarkably good preservation of mucosa, especially considering the delay in commencing the autopsy. The glands are tall and well supplied with oxyntic cells. The muscularis is thin. Between the muscularis mucosae and the true muscularis there is a prominent, now folded, aneurysm. It is seen to arise on one side from a wide artery and then pushes up the mucosa, which is destroyed over an extent of 3 to 4 mm. This point corresponds to a large plug of partially organized thrombus, blocking an extensive rupture in the most superficial part of the aneurysmal wall. The immediately neighboring mucosa is heavily infiltrated with lymphocytes. No other inflammatory reaction is seen in the slide.

The aneurysm is seen to have the structure of a muscular artery (Fig. 3), with three distinguishable layers. A well-developed muscular coat is present in all parts of the circumference. In several areas, the muscle fibers have pyknotic nuclei and the nuclei are seen to be much reduced in number; in such places there is a hyalinization in which the identity of the individual fibers is lost. Here, too, minute empty vacuoles are present. Masson's trichrome stain shows that in such areas only a few red-staining muscle fibers are present, the remainder being replaced by a structureless, green fibrous tissue. At the two ruptured ends of the aneurysm, the muscle coat is represented almost to the very end, where it has been converted into connective tissue. The intima is thickened nearly everywhere, but the degree of this change varies from place to place, being by no means maximal at the site of rupture. Van Gieson-elastic stain shows that the internal elastic lamina is always represented but is very frequently frayed or reduplicated. It, too, is present at the termination of the broken ends of the vessel. A few small spaces are seen in the intima, but these do not have the shape of cholesterol clefts. There are no calcium deposits.

## COMMENT

Including Bianchedi's own case, but not his miscellaneous cases from the literature,

the number of ruptured gastric aneurysms collected is 16, and the present report adds a 17th case.

All reports in which microscopy has been performed have shown what the authors describe as sclerotic or arteriosclerotic changes. Generally, the presence of a medial coat has been noted, and the present case is no exception, although scattered areas of degeneration have been commented on. Forbus,[29] discussing the origin of congenital miliary aneurysms of the superficial cerebral arteries, regards disappearance of the internal elastic lamina as a relatively early event, even before the persistent saccular dilatation forms. He cites Busse † and Emrich ‡ in their discussion of stretching (*Dehnung*) aneurysms. They regard simple dilatation as the cause of the degeneration of the muscularis and the elastica. The intimal hyperplasia is compensatory at the site of weakening. Forbus states that congenital medial defects have not been described in the mesenteric or the splanchnic vessels and defends the opinion that congenital aneurysms arise from areas of defect in the media of the parent vessel. In a paper on this topic, Forster and Alpers,[32] examining eight cases of cerebral aneurysm, believed that the primary abnormality is the medial defect at the point of bifurcation and that the changes in the elastica are secondary to the formation of the aneurysm.

The authorities cited with reference to the present topic have not seen a congenital gastric aneurysm, with the exception of Scheidegger, whose case had multiple varices throughout the gastrointestinal tract Although Mallory declared that he could not tell a congenital from an acquired aneurysm, it would appear that the presence of all three coats speaks in favor of an acquired lesion.

*Analysis of Cases of Gastric Aneurysm Reported in the Literature*

| Case | Age | Race | Sex | Pathology | Outcome |
|---|---|---|---|---|---|
| Jejunum | | | | | |
| Rathmell et al. | 29 | W | F | Thinning of media; no arteriosclerosis | Died |
| Levine and Valk | 18 | N | F | Absent elastica; medial degeneration; intimal fibrosis | Lived |
| Stomach | | | | | |
| Gallard | 20 | W | M | Trace arteriosclerosis | Died |
| | 51 | ... | .. | Not stated | Died |
| Sachs | 70 | W | M | Medial destruction at dome of aneurysm | Died |
| Hirschfeld | 38 | W | M | Aneurysm + hemophilia + alcoholism | Died |
| Hoelzer | 84 | W | M | Marked medial degeneration, intimal proliferation at dome with loss of elastica | Died |
| Scheidegger | .. | ... | .. | "Congenital" aneurysm with multiple varices in GIT | ..... |
| Bianchedi | 40 | W | M | Diffuse elastic disorganization and intimal proliferation; local medial destruction | Died |
| Heuer | .. | ... | .. | ..... | Died |
| Mallory | 55 | W | M | Arteriosclerosis | Lived |
| Donaldson and Hamlin | 55 | W | M | Arteriosclerosis | Lived |
| | 59 | W | M | Sclerotic | Died (complications) |
| | 64 | W | M | ..... | Died |
| Millard | 46 | W | M | Diffuse intimal proliferation, splitting of elastica and medial degeneration | Died |

All the cases cited have shown a range of from a trace of, to severe arteriosclerosis, the former only being mentioned in the case of Gallard's patient, who was 20 years old (Table). The distribution of the lesion in males is also to be noted. The only cases of this type reported in females are those in the jejunum, in one of which the patient, only 18, showed arteriosclerotic changes. The Table also shows that one patient of the gastric cases and one of the jejunal cases survived the condition.

While certain of the authors comment on abnormal findings being restricted to that

† Busse,[30] cited by Forbus.[29]

‡ Emrich,[31] cited by Forbus.[29]

part of the aneurysm adjacent to the rupture and ulceration, such a statement cannot be made in the present case, where the uniformity of the change throughout the circumference has been described. There are thus no grounds for claiming this case as congenital. Moreover, for the same reason, it cannot be held that a mucosal erosion was the primary lesion. The survival of the elastica, albeit with splitting, points to a slowly developing lesion.

Finally, the fact that Donaldson and Hamlin could report three cases, of which one patient lived while another died of postoperative causes, suggests that the condition may be more frequent than is suspected.


## SUMMARY

A 16th authenticated case of ruptured gastric aneurysm is reported.

The lesion was arteriosclerotic and in a man, as in practically all other cases.

The high fatality rate indicates the need for surgical consultation in patients with gastric hemorrhage which continues or recurs.

The moral is drawn that, in stomachs in which no point of bleeding can be found, injection of the vessels may provide the answer.

A complete review of the literature is attempted.


Dr. Philipp R. Rezek and Dr. J. Miale provided translations; Mr. W. Atkinson supplied photographs, and Dr. W. Lindau granted permission to use this case.

## ADDENDUM

After completion of this paper, a further case of Albrich's,[33] from Vienna, came to light. This was a woman in her 50's with severe hematemesis. She died nine days after gastrotomy failed to reveal a bleeding point. Autopsy succeeded in showing an aneurysm of cherry-stone size, 9 cm. below the cardia on the posterior wall. The stomach contained several mucosal scars, considered to be healed ulcers, and Albrich considered that the aneurysm might have occurred at such a site. He found that the vascular dilatation had a thickened elastica externa, intimal cushions narrowing the lumen, and fibrinoid necrosis at the edges. The last finding was believed to be due to bacterial necrosis. The author called the aneurysm a false one, as the sac consisted of laminated fibrin, not vessel wall. No pictures accompany the article.

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# Hürthle-Cell Tumors of the Thyroid

LAWRENCE W. GARDNER, M.D., Detroit

Hürthle cell tumors of the thyroid still remain a controversial subject, even though many isolated case reports appeared in the older medical literature and larger reviews have been published within the last five years. At present, the foremost problems are those of benignity or malignancy and treatment. With a thorough clinicopathologic study of 46 Hürthle cell tumors, our compiled experience seems worthy of attention.

## NOMENCLATURE AND INCIDENCE

The actual incidence of these uncommonly recorded tumors cannot be correctly established owing to lack of unanimity in nomenclature and classification. The following terms have been used: Getzowa's struma; Baber cell tumor; struma postbranchialis; *kleinalveolare, grosszellige Struma;* Langhans' struma, adenoma, or tumor; Hürthle cell adenoma, carcinoma, or tumor; large-cell, small acinar tumor of Langhans; parafollicular cell adenoma; encapsulated acidophile cell adenoma or adenocarcinoma; oxyphil-cell adenoma; small alveolar and large-cell adenocarcinoma; eosinophilic large-celled tumor of the thyroid, and adenoma or carcinoma with partial or complete oncocytic transformation. With these synonyms, 194 Hürthle cell tumors are noted in the world's medical literature since these new growths were first described by Langhans [26] in 1907.

However, as a whole, pathologists lost sight of these peculiar tumors, and some neoplasms are erroneously designated as adenoma malignum, fetal adenoma, Wurchende's struma, and various forms of unusual carcinomas of the thyroid gland. In 1926 Kolodny [24] recorded a case of "hypernephroma of the thyroid," in which evidence of a primary renal tumor was lacking; careful examination of the patient 11 months later revealed excellent health without metastases, recurrence in the neck, or demonstrable neoplasm in the kidneys. A consultant pathologist regarded the tissue as a "local overgrowth of alveolar cells with hydropic degeneration." This tumor may represent a form of Hürthle cell new growth. In a series of thyroid neoplasms documented by Wilson,[35] of the Mayo Clinic, a great variety of histopathological patterns were grouped under benign and malignant adenomas. At least one photomicrograph is suggestive of a Hürthle cell tumor. In his monograph "Surgical Pathology of the Thyroid" Hertzler [20] describes fetal adenomas simulating adrenal cortex or hypernephromas, which may represent Hürthle cell adenomas or carcinomas. Ewing [11] cites two cases of Hürthle cell tumor and presents an excellent photomicrograph. Other instances could be cited.

Therefore, the rarity of Hürthle cell tumors is misleading in the collected reports. Undoubtedly, a review of thyroid pathology in the large clinics, particularly in geographic areas of high endemicity of nodular goiters, will disclose additional Hürthle cell tumors.

Recent textbooks of pathology (Ackerman,[1] Bell,[7] Boyd,[8] Karsner,[23] Moore,[28] and Willis [34]) include adequate descriptions of Hürthle cell tumors, so that these entities may be correctly classified in the future.

Even though unfortunate (Willis [34]), the preservation of the general heading of Hürthle cell tumors, with subdivision into Hürthle cell adenoma and Hürthle cell carcinoma, seems appropriate on the basis of prolonged usage and our experience with these new growths. Such a statement will not

From the Division of Laboratories, Mount Carmel Mercy Hospital.

receive uniform acceptance, since the American Cancer Society,[3] in 1951, abandoned these terms, replacing them with "Hürthle cell adenocarcinoma" and "Hürthle cell carcinoma." Thus, a malignant nature is indicated for these tumors—a point of distinct controversy.

## ORIGIN

In a chronological order, Table 1 outlines the theories of origin, with its obvious change of thought through the years. At present, the majority of writers favor a physiopathological

TABLE 1.—*Origin of Hürthle Cell Tumors*

| | |
|---|---|
| **Interfollicular cell theory** | |
| Baber | 1881 |
| Langendorff | 1889 |
| Hürthle | 1894 |
| Ewing | 1928 |
| Ebert | 1933 |
| Bakay | 1948 |
| **Ultimobranchial body theory** | |
| Getzowa | 1907 |
| Langhans | 1907 |
| **Ectopic oxyphilic cell of Welsh** | |
| Eisenberg-Wallerstein | 1932 |
| **Oncocyte theory** | |
| Hamperl | 1936 |
| Hamperl | 1950 |
| **Physiologic alteration in thyroid epithelium** | |
| Wegelin | 1926 |
| Wilensky-Kaufman | 1938 |
| Harry | 1941 |
| Morrow | 1945 |
| Lennox | 1948 |
| Friedman | 1949 |
| Chesky-Dreese-Hellwig | 1951 |
| Horn | 1954 |

alteration of thyroid epithelium rather than an anatomic basis. As emphasized by Lennox,[27] a functional origin is substantiated by the presence of small and large groups of so-called Hürthle cells in all forms of thyroid disease. From a strict oncological standpoint, these elements should be designated Askanazy [4] cells, since Askanazy first described this particular cell in humans as opposed to interfollicular cells of Baber,[5] found only in animals.

The "normal" thyroid of women contains numerous Askanazy cell groups as compared to the gland of the male, and, in general, corresponds with the much greater incidence of Hürthle cell tumors in the female. In 100 consecutive female postmortem examinations at our institution, Askanazy cell groups were found in 31% of thyroid glands without other histopathological changes. In a similar 100 consecutive male postmortem examinations, only 3% of thyroids disclosed Askanazy cell groups. These findings suggest an endocrine factor as a possible mechanism of physiologic alteration of the thyroid epithelium, particularly since 25 of the 31 cases with Askanazy cell change were found in women over 50 years of age. Parmley and Hellwig [30] have postulated a series of events, initiated by loss of ovarian function, leading to lymphadenoid goiter in which numerous Hürthle cell groups are noted. A similar mechanism may be involved in the genesis of Hürthle cell adenomas and carcinomas.

The oncocytic theory of Hamperl * postulates a degenerative change in the parenchymal epithelium of an organ, resulting in large polyhedral cells with voluminous acidophilic granular cytoplasm. Such cells have been found in the thyroid, parathyroids, pituitary, adrenals, salivary glands, pancreas, liver, Fallopian tubes, testes, and bronchi. Hamperl * proposes the term "oncocytoma" for tumors composed of these cells. Although fundamentally similar to the American viewpoint in relation to Hürthle cell tumors, the terminology has not been accepted in our country.

## MATERIAL AND METHODS

Forty-six cases of Hürthle cell tumors † were studied in detail, and, since interest in this subject began in 1940, extreme care was used in the preparation of the material. Other adenomas and carcinomas showing some degree of Hürthle cell change were purposely omitted from this study, since the basic processes represented other entities than the homogeneous acidophilic Hürthle cell tumor.

Clinically, it was possible to follow these patients for significant periods, and their courses included thorough medical examina-

* References 17 and 18.

† A portion of this material was made available through Dr. Plinn F. Morse.

tions by the attending physicians. In 10 cases recent roentgenological study of the skeleton was performed, and, in one instance, tracer doses of radioactive iodine[131] were employed.

Tissue sections varied from 4 to 18 per case, and all microscopic preparations were stained with hematoxylin and eosin, Van Gieson's, Masson's trichrome, Mallory's aniline-blue, and silver impregnation for reticulum (Foot's modification of Bielschowsky's method). In addition, the following stains were used where possible: Best's carmine stain for glycogen, periodic acid-Schiff (PAS) reaction, and Sudan IV for lipid substance.

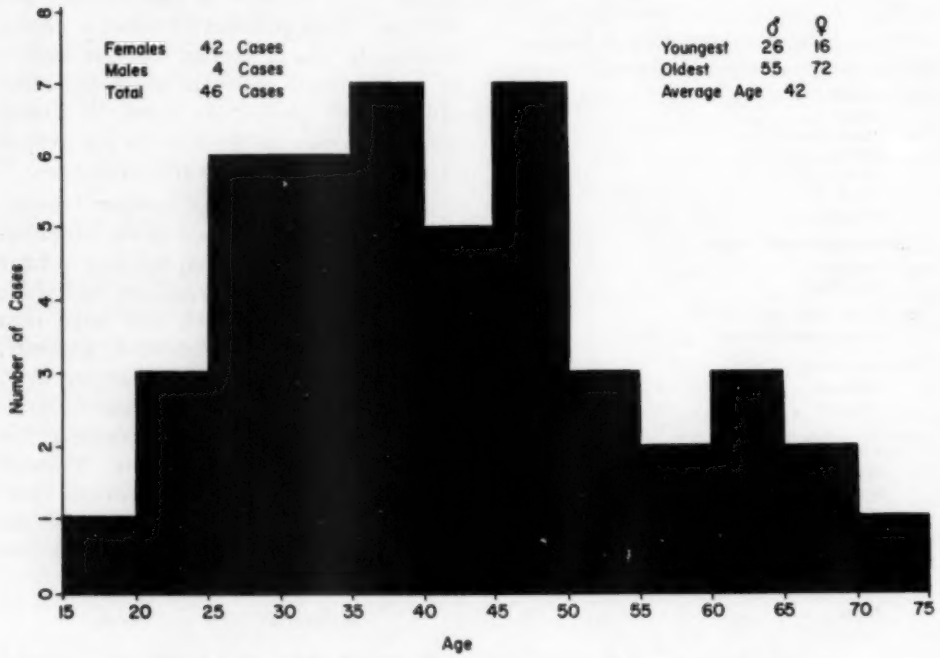



Fig. 1.—Sex and age distribution in 46 Hürthle cell tumors.

## INVESTIGATIONAL DATA

*Clinical Summary.*—In this study 42 cases were observed in women and 4 were noted in men, representing a ratio of 10:1. In a review of 40 cases of Hürthle cell cancer by Frazell and Duffy,[14] a female predominance of 2:1 was noted; the collected survey of Morrow [29] revealed a 6:1 ratio; and, in the frank carcinomatous group of these tumors, Horn [21] presents a ratio of 2.5 females to 1 male.

The average age in our series was 42 years. In the female group the ages ranged from 16 to 72, while the men varied from 26 to 55 (Fig. 1). Our average age is somewhat lower than that presented in other large reviews. In the paper of Morrow [29] (23 cases) 70% of the patients were over 40 years of age, and Goldenberg [16] states that 82% of his series (22 cases) were above this age. Chesky and co-workers [9] (25 cases) and Frazell and Duffy [14] (40 cases) independently cite an average age of 50.

While these neoplasms are commonest in middle life, Symmers [31] and Morrow [29] recorded congenital Hürthle cell tumors, the former occurring in a white girl, aged 6 weeks, and the latter occurring in a white girl, aged 8 weeks.

The chief complaints in our series are indicated in Table 2.

TABLE 2.—*Summary of Chief Complaints*

| | Cases, No. |
|---|---|
| (*a*) Palpable, painless mass in neck | 37 |
| (*b*) Gradual increase in size of neck | 4 |
| (*c*) Dysphagia | 2 |
| (*d*) "Nervousness" | 1 |
| (*e*) Choking sensation | 1 |
| (*f*) Mass discovered in routine examination | 1 |

The duration of the thyroid mass varied from 1 month to 35 years. The average duration was seven years. Secondary symptoms of dysphagia, dyspnea, or tightness in the neck were given by 60% of the patients.

A previous history of hyperthyroidism was given in five instances, but evidence of this entity was lacking in all cases. One patient had had an operation for "goiter" 10 years prior to the present admission. Association with bone or joint manifestations was not demonstrated in any case. Palpable cervical lymphadenopathy was absent.

The laboratory studies (compiete blood count, urinalysis, and serologic tests) were not remarkable. Basal metabolic studies were performed on 28 patients, and the results were within the normal limits. Cholesterol determinations upon 16 serums failed to disclose significant alteration. Four serum iodine estimations revealed normal values. Except for one substernal mass and four tracheal shifts, routine chest roentgenograms were not enlightening. Undoubtedly, associated esophageal studies would have disclosed pressure defects in some instances.

The locations of the 46 Hürthle cell tumors are indicated in Table 3.

*Gross Anatomic Pathology.*—In this series 38 of the Hürthle cell tumors were solitary, spheroid or ovoid, well-circumscribed and encapsulated new growths (Fig. 2), which were easily enucleated from the adjacent structures. Grossly, the capsules were intact. The smallest tumor measured 1.5 by 1.0 by 1.0 cm.; the largest mass measured 12.0 by 10.0 by 9.0 cm. The latter weighed 374 gm. In this series the average weight was 71 gm. The cut surfaces were light tan, gray-yellow, yellow-pink, or yellow-gray in color and moderately firm in consistency. Variegation resulted from hemorrhagic extravasations, ischemic necrosis, and occasional radiating bands of connective tissue. However, except in one case, a central hyaline scar was absent. Between these zones, the new growth presented a homogeneous appearance. Calcium deposits were not noted. In five cases, a cystadenomatous structure (Fig. 3) was observed, as noted by the presence of unilocular or bilocular cysts (largest 4.0 cm. in diameter), which were filled with a brown, watery or gelatinous fluid.

Fig. 2.—Gross specimen showing an encapsulated spheroid tumor (5.5 by 4.5 by 3.5 cm.) with homogeneous firm yellow-tan cut surfaces.

In three cases, two separate encapsulated nodules were found in the same lobe. In two instances, the primary pathologic change was that of lymphadenoid goiter.

*Histopathology.*—The characteristic cells of these tumors were polyhedral elements measuring 15$\mu$ to 25$\mu$ in diameter. The cytoplasm was abundant, densely eosinophilic,

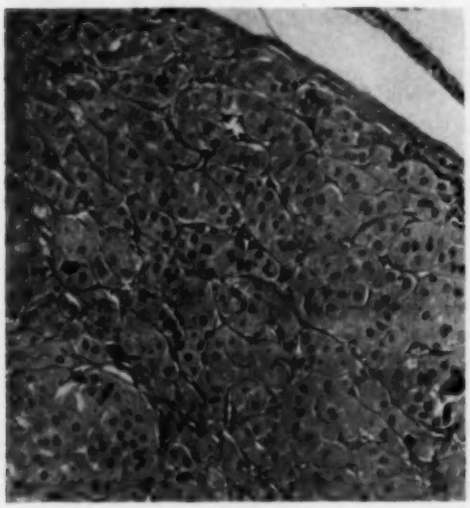
TABLE 3.—*Locations of Hürthle Cell Tumors*

| | Cases, No. |
|---|---|
| Right lobe of thyroid gland | 19 |
| Left lobe of thyroid gland | 23 |
| Isthmus of thyroid gland | 3 |
| Retrosternal site | 1 |
| Lingual or pyramidal sites | 0 |

Fig. 3.—Gross specimen of a cystadenoma with a yellow-gray tumor (4.0 by 3.5 by 3.0 cm.) attached to one side of cavity.

opaque, and finely granular; occasionally, it was foamy and vacuolated. Usually the cytoplasmic membranes were unusually distinct. The nuclei, often eccentrically placed, were vesicular, and the chromatin was irregularly distributed. Definite mitotic figures were not observed. A minimal-moderate degree of anisocytosis, anisokaryosis, and hyperchromasia was noted in this series. In a few instances, nuclear pyknosis was conspicuous.

Fig. 4.—Trabecular Hürthle cell tumor; reduced ⅔ from mag. × 200.



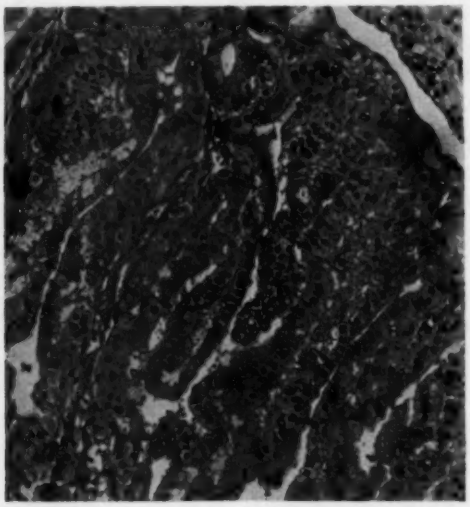
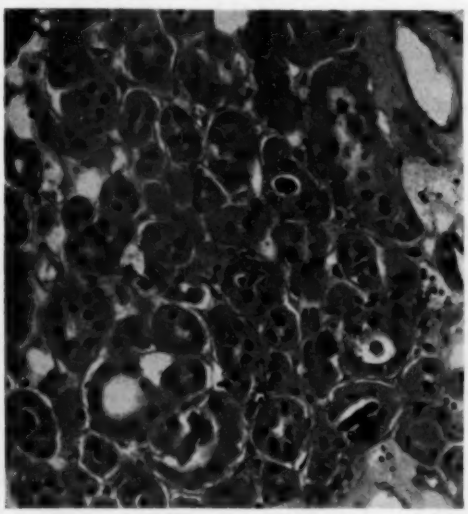


Fig. 5.—Tubular Hürthle cell tumor; reduced ⅔ from mag. × 200.

In general, these peculiar acidophilic cells were supported by a delicate vascularized connective tissue dividing the tumor into various-sized lobules. Chesky and co-workers [9] have suggested four forms of Hürthle cell tumors based upon architectural arrangement: trabecular, small alveolar, large follicular, and papillary patterns. Using this classification, 23 tumors were predominantly trabecular (Figs. 4 and 5), 12 were small

Fig. 6.—Small alveolar Hürthle cell tumor; reduced ⅔ from mag. × 220.



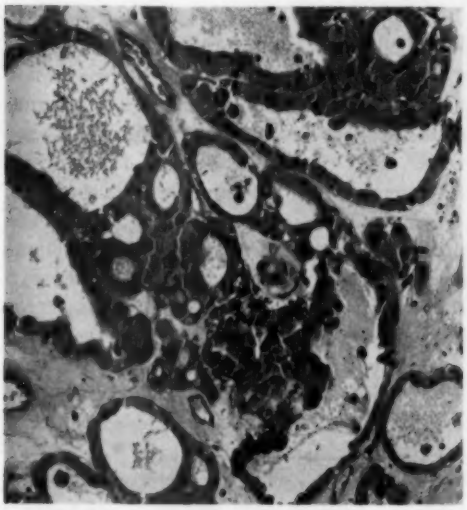


Fig. 7.—Large follicular Hürthle cell tumor; reduced ⅔ from mag. × 220.

alveolar (Fig. 6), 6 were follicular (Fig. 7), and 5 were papillary in type (Figs. 8, 9, and 10). In the alveolar and follicular varieties, some vesicles were partially filled by a thin colloid, while others were empty. Since only 50% of each type were uniform in cellular arrangement, mixed varieties were rather common.

Fig. 8.—Papillary Hürthle cell tumor; reduced ⅔ from mag. × 30.

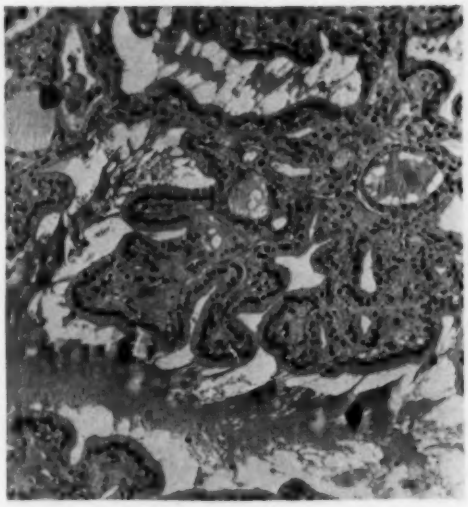
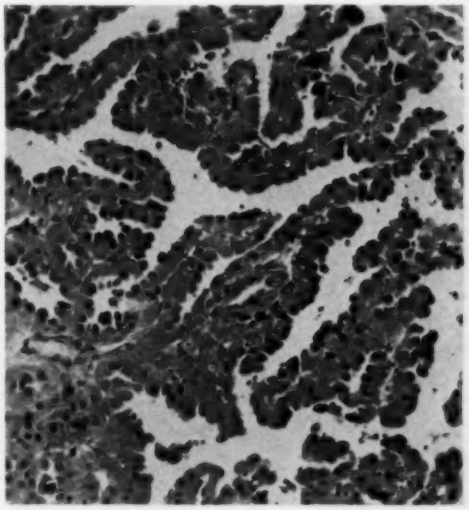


Fig. 9.—Papillary Hürthle cell tumor; reduced ⅔ from mag. × 175.

In 45 cases the histologic features were uniform, without evidence of significant anaplasia, or vascular or capsular invasion, and a diagnosis of Hürthle cell adenoma was made.

In one instance, anaplasia and capsular invasion necessitated a diagnosis of Hürthle cell carcinoma ("Treatment," below).

Fig. 10.—Papillary Hürthle cell tumor; reduced ⅔ from mag. × 200.



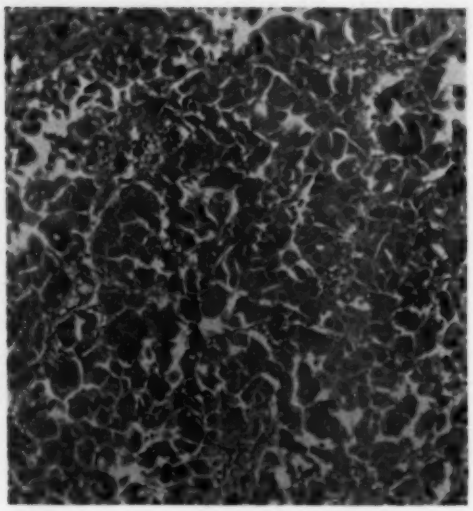


Fig. 11.—Hürthle cell carcinoma showing anaplasia (Grade III); reduced ⅔ from mag. × 175.

The adjacent thyroid parenchyma was the site of minimal epithelial hyperplasia in 20% of our cases, but positive clinical evidence of hyperthyroidism was lacking. Minute aggregations of lymphocytes, occasionally with germinal centers, were noted in the thyroid parenchyma in 53% of our cases. Only two cases were associated with lymphadenoid goiter.

Fig. 12.—Hürthle cell carcinoma showing invasion of capsule; reduced ⅔ from mag. × 150.

*Treatment.*—The treatment consisted of lobectomy in 23 cases and subtotal thyroidectomy in 22 instances.

The final case presents a rather interesting course and represents the only death in our series. In 1930, a "resection" of a Hürthle cell adenoma (trabecular type similar to Fig. 4) in the lower pole of the right lobe was performed. In 1941, recurrence at the operative site resulted in another "partial resection"; a portion of the mass was retrosternal in location. Histopathologic diagnosis was "recurrent Hürthle cell adenoma of trabecular type" (Fig. 5). In 1943, a subtotal thyroidectomy and removal of a substernal mass

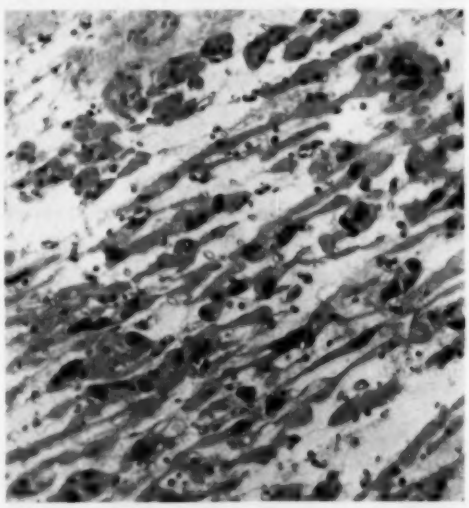


Fig. 13.—Hürthle cell adenoma showing marked pyknosis; reduced ⅔ from mag. × 200.

was performed. The combined weight of the four resected masses was 275 gm., and two-thirds of the tumor was posterior to the upper sternum, resulting in compression and displacement of the trachea, which was adequately shown on the roentgen films. At this time, the patient was 60 years of age and had a known severe degree of arteriosclerotic heart disease. Auricular fibrillation terminated in acute congestive failure four days postoperatively.

Permission for postmortem examination was granted, and complete roentgenological study of the skeleton was performed; sus-

picious metastatic areas were not demonstrated. A careful postmortem examination disclosed only a minute amount of new growth beneath the manubrium. While the previous surgical specimens revealed the uniform features of Hürthle cell adenoma, histopathological study of the last surgical and postmortem tissues disclosed considerable anaplasia (Grade III) with capsular invasion (Figs. 11 and 12). Definite tumor emboli were not demonstrated in the vascular channels. If lobectomy had been performed as the initial operation, this fatal course may have been prevented.

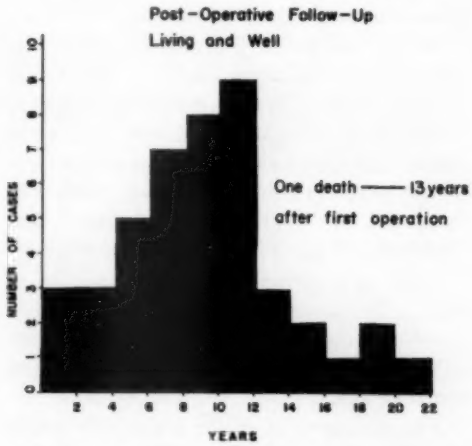



Fig. 14.—Follow-up studies on 46 cases of Hürthle cell tumors.

Radon insertion, radical neck dissection, or postoperative irradiation therapy was not performed in this series.

*End-Results.*—Figure 14 indicates our postoperative follow-up. Forty-five patients are living and well, for variable periods. Only cases with a minimum of a two-year course are included in this series. Since 35 cases (76%) have been followed for more than six years and 28 cases (60%) observed for over eight years, our figures seem significant. As previously indicated, each patient has received a recent thorough medical examination and, with the exception of one postoperative death, these patients are apparently free of demonstrable metastases or recurrent disease.

Therefore, in this series we believe that the majority of these new growths are benign tumors but recognize that a definite growth potential is present, which occasionally may develop into a frank carcinomatous process.

## COMMENT

The benignity or malignancy of Hürthle cell tumors cannot be answered in an unequivocal manner. Diametrically opposite viewpoints require careful thought for resolution of differences.

Apparently Frazell and Duffy [14] consider all tumors in this category as Hürthle cell cancer, and, in a series of 40 cases, skeletal metastases were demonstrated in 12 instances and cervical lymph node involvement in 10 cases. In addition, 12 patients died of the disease, with regional and distant metastases. While more radical procedures were performed on some patients, they favor complete excision of the involved lobe and isthmus in primary cases of Hürthle cell cancer, and combined radical thyroidectomy and neck dissection in recurrent or residual tumor of this type.

In a series of 75 Hürthle cell tumors, Horn [21] classified 14 as involutional nodules, 27 as adenomas, and 34 as carcinomas. In the latter group, 12 cases were designated as malignant adenoma without incontrovertible proof of malignancy, thus leaving 20 cases as frank carcinomas with invasive growth (usually histological) or metastases in 10 instances. Horn [21] emphasizes the difficulties encountered in the classification of these tumors and the uncertain growth capacity of Hürthle cells.

In the collected series (23 cases) of Morrow,[29] 8 were diagnosed as carcinoma (2 Langhans and 1 Haagensen) with distant metastases. Chesky and co-workers [9] (25 Hürthle cell tumors) report that 20 patients are living without evidence of disease for one and one-half to nine years after thyroidectomy. Two patients showed evidence of recurrent tumor and three patients died without positive evidence of metastatic malignancy. In their opinion, removal of the involved lobe

was considered sufficient for solitary Hürthle cell tumors. If palpable cervical lymph nodes were present, radical neck dissection was warranted, with subsequent postoperative irradiation. Goldenberg [16] (22 Hürthle cell carcinomas) prefers local surgical excision, preferably lobectomy with radical neck dissection reserved for invasive carcinoma or residual or recurrent tumor. Only one patient died with this condition, and one is living, with evidence of recurrence.

Perhaps the difference of opinion may be partially resolved by strictly limiting the term "Hürthle cell tumor," with its subdivisions of adenoma and carcinoma, to those new growths composed wholly of cells with abundant granular eosinophilic cytoplasm. Other adenomas and carcinomas showing some degree of Hürthle cell change should be omitted, since the basic processes represent different entities from the homogeneous acidophilic Hürthle cell tumor. Usually, the carcinomatous lesions will reveal hyperchromicity, anaplasia, mitotic figures, and alteration of the nucleocytoplasmic ratio, but the granular eosinophilic cytoplasm should be retained. Thus, the majority of highly undifferentiated carcinomas of spindle, giant-cell or mixed varieties must necessarily be excluded.

Accepting the theory that the Hürthle cell is merely a physiopathologic variant of thyroid epithelium, these cells are nonspecific elements and may be found in all types of thyroid glands. In adenomas and carcinomas this change may be complete, so that the identity of the original tumor cannot be established. For this group the term "Hürthle cell carcinoma or adenoma" seems warranted. Even here, some cases must be designated merely as "Hürthle cell tumor."

As suggested by Lennox,[27] we believe that many Hürthle cell tumors arise from fetal adenomas. Thirty-six of our cases belong to this group, but we do not concur with the postulated frequent malignant character. Eight cases were associated with adenomatous goiters and two cases with lymphadenoma.

From our study the Hürthle cell change is associated with a decreased growth capacity and a relatively favorable prognosis.

The degree of benignity or malignancy depends upon the nature of the original process in which the degenerative eosinophilic change occurred. In this regard, many sections must be studied from each tumor with particular reference to its histopathological features and vascular and capsular invasion.

## SUMMARY

The clinicopathologic features of 46 Hürthle cell tumors have been presented. Forty-five new growths are designated as adenomas and 1 as a carcinoma.

Beside the pathologic characteristics, the benign nature of these tumors is borne out, to some extent, by the follow-up study, in which 45 patients are living and well, without recognizable evidence of the disease.

The term "Hürthle cell tumor," with subcategories of "Hürthle cell adenoma" and "Hürthle cell carcinoma," should be retained.

Only new growths composed wholly of cells with abundant acidophilic cytoplasm should be classified as Hürthle cell tumors.

The nomenclature, incidence, and origin are reviewed.

The treatment of choice is complete lobectomy in primary Hürthle cell tumors. If palpable cervical lymphadenopathy is present, total thyroidectomy with neck dissection is warranted.

In our opinion, Hürthle cell change is associated with a decreased growth capacity and, if the process is complete, a favorable prognosis in most cases.

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# Diffuse Interstitial Fibrosis of the Lungs

CHARLES POKORNY, M.D.
and
C. A. HELLWIG, M.D., Halstead, Kan.

In 1935 Hamman and Rich described three cases of acute diffuse interstitial fibrosis of the lungs which presented distinctive clinical and pathological features. They added one other case [2] nine years later, and, since then, 27 * cases have been reported in the literature.

According to Hamman and Rich, the process begins insidiously as a wide-spread inflammation that involves all lobes of both lungs diffusely. The alveoli are filled with albuminous fluid, relatively few polymorphonuclear leucocytes, and a little fibrin. Very shortly after the onset of pulmonary inflammation there occurs a marked proliferation of connective tissue, which greatly thickens the alveolar walls and obliterates many of the air sacs. The fibrosis disturbs the relation of the alveolar capillaries to the air spaces and results in ever-increasing dyspnea. As pulmonary fibrosis increases and spreads, the pressure in the pulmonary artery is increased and the right ventricle becomes dilated and hypertrophied. Sooner or later symptoms of congestive heart failure appear. The following case which we had the opportunity to study fulfills, in our opinion, the criteria of Hamman and Rich's syndrome.

## REPORT OF A CASE

W. F., a 72-year-old white man, was hospitalized July, 1951, with evidences of a hydrocele and heart trouble. At the time of the interview the patient was irrational and for that reason his history was not considered reliable. He stated he had had heart trouble for 25 years. In the past month he had arrived from the West Coast to Eastern Colorado and had become extremely short of breath. With this he had a constant hacking cough and at times was slightly cyanotic. His legs had begun to swell badly during the last month. He had come to the clinic by bus and felt that during the ride he had taken cold, as now his chest felt tight and his cough was severer.

*Physical Examination.*—The patient was so dyspneic that he could not lie down on the examining table. Blood pressure was 120/95, pulse 120 and irregular. There was a mild cyanosis present. The heart was not enlarged to percussion. A pulse deficit was present. The first chest examination revealed no areas of dullness to percussion, and no rales were heard. The abdomen was negative. The patient had a large left hydrocele. There was pitting edema of the lower extremities. Urine analysis was pH 4.5, albumin trace, sugar negative, microscopic findings negative. Hemoglobin 12.5 gm. per 100 cc., RBC 3,900,000, WBC 7600. The Kline test was negative.

The patient had a stormy course in the hospital; he was critically ill and so confused that he was difficult to control. He appeared to be in severe cardiac failure. He received digitalis, a mercurial diuretic, sedatives, and oxygen without gaining relief. In spite of a 10-lb. (4.5-kg.) weight loss, which improved his edema, this patient showed marked dyspnea, even when sitting in a chair. He became irrational and complained of smothering.

During his hospitalization, a few moist rales were heard in the bases of both lungs but were minimal and out of proportion to his respiratory distress. While the cough was quite constant, only a small amount of white mucoid sputum was expectorated. Along with treatment of his heart failure, 400,000 units of penicillin with 0.5 gm. of streptomycin was given daily. The patient's condition became progressively worse and he died on the eighth hospital day.

## AUTOPSY FINDINGS

Permit was obtained for examination of thoracic cavity only.

*External Examination.*—Body of a white man about 75 years old, 5 ft. 6 in. (167.64 cm.) in length, weight about 135 lb. (61.2 kg.). Dark-

From the Hertzler Clinic and Hertzler Research Foundation.

Read before the Section on Pathology and Physiology at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 22, 1954.

* References 3 through 18.

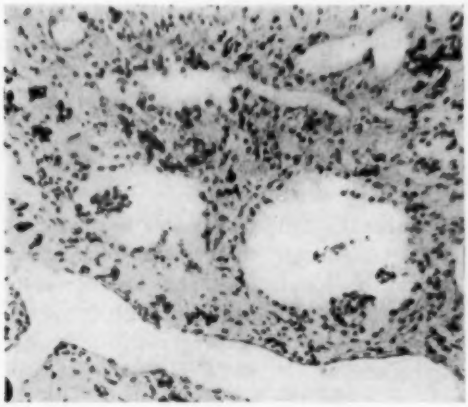
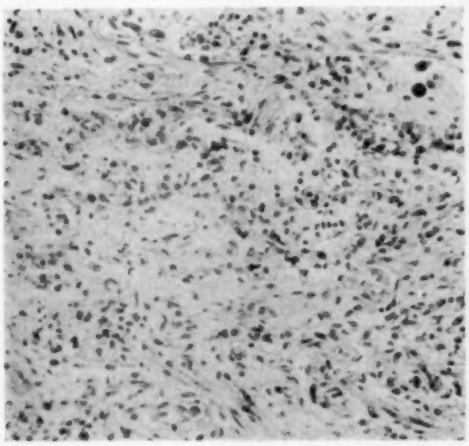


Fig. 1.—Thickening of interalveolar septa by fibrous tissue. No organization of exudate in alveolar lumens. Reduced 1/9 from mag. × 140.

bluish complexion of hands and face. Scanty white hair. The body was emaciated. There was moderate edema of both ankles. A large left hydrocele was found. Several hematomas were found in the right cubital fossa. The body was arterially embalmed.

The cartilages of the ribs were soft and brownish in color. The thymus gland was atrophic. The left pleural space contained about 100 cc. of clear fluid, and the right pleural space, about 200 cc. of fluid. There were dense fibrous adhesions over the posterior surface of both lower lobes. Anthracosis was pronounced in both lungs. The right lung weighed 900 gm. and the left, 660 gm. The lungs were firm and did not collapse. The cut surface of both lungs revealed a diffuse patchy consolidation, with a firm nodular appearance and a reddish-brown color. In some areas there were more extensive solid smooth

Fig. 2.—Diffuse fibrosis of lung tissue, alveoli obliterated; reduced 2/7 from mag. × 140.



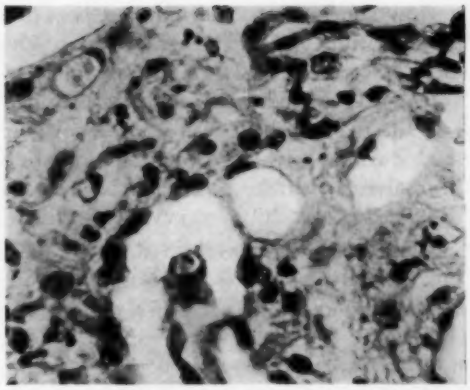
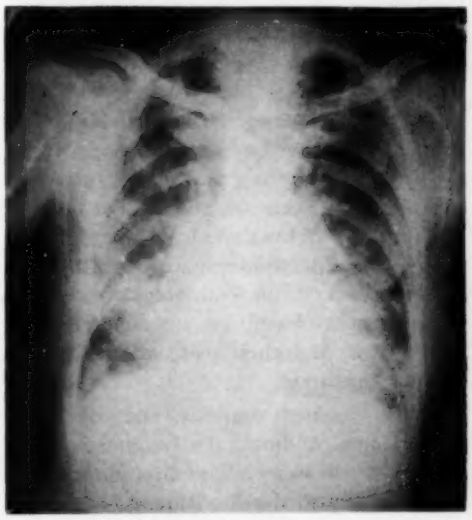


Fig. 3.—Thick interalveolar septum with fibroblasts, lymphocytes, and histiocytes; reduced 2/5 from × 580.

gray portions, which were firmer than the remaining lung tissue. The bronchi contained a clear mucoid material but no pus. Several moderately enlarged lymph nodes were present at the hilus of each lung. The heart weighed 490 gm. The wall of the left ventricle measured 1.4 cm. in thickness; that of the right ventricle, 8 mm. The right ventricle was markedly dilated. There was some calcification in the wall of the left coronary artery.

*Microscopic Examination.*—In all lobes of the lungs an extensive interstitial fibrosis was noticed. The alveolar septa were wide and were composed of a more or less cellular fibrous tissue. The alveolar capillaries were

Fig. 4.—X-ray at time of admittance, showing areas of infiltration.



narrow and scanty. The lining cells of the alveolar walls were often high cuboidal. The alveoli were decreased in number; some contained coagulated serum, and some, round cells. No giant cells were noticed. Few lumina contained fibrous plugs of organized intra-alveolar exudate. Several bronchioles contained neutrophilic leucocytes.

While most fibrous septa did not show inflammatory cells, in several areas they contained lymphocytes, plasma cells, eosinophilic cells, and fibroblasts. Macrophages with hemosiderin pigment were noticed in some of the septa and in the lumen of some alveoli. The pulmonary arterioles showed thickening of the intima and media. Stains for bacteria were negative. The paratracheal and hilar lymph nodes showed moderate hyperplasia with deposition of carbon. In the myocardium small areas of fibrosis were evident. The anatomical diagnosis was diffuse interstitial fibrosis of the lungs; dilatation and hypertrophy of the right ventricle (cor pulmonale); focal myocardial fibrosis.

TABLE 1.—*Symptoms and Findings in 27 Cases of Reported Interstitial Pulmonary Fibrosis*

| | |
|---|---|
| Progressive dyspnea | 27 |
| Cough | 26 |
| Cyanosis | 22 |
| Fever | 8 |
| Clubbing of fingers | 6 |
| Hemoptysis | 5 |
| Polycythemia | 4 |

## COMMENT

*Clinical Symptoms* (Table 1).—The syndrome, which may be either acute or chronic, is manifested by dyspnea, cough, and cyanosis. The dyspnea is progressive in type, and the physical findings of the lungs are surprisingly few, considering the patient's distress. Other clinical findings found are hemoptysis, clubbing of the fingers, fever, weight loss, and chest pain, although these are not consistent.

While dyspnea, cyanosis, and cough are the principal findings, the cough is usually dry and troublesome. The disease may progress rapidly to death within a few weeks, or it may manifest itself as a chronic disease with intermittent exacerbations. In either form, the disease usually ends with cyanosis and marked respiratory insufficiency terminating in death.

## DIAGNOSIS

We believe, as do Peabody and co-workers,[13] that this syndrome cannot be diagnosed without histologic study; that Callahan's [11] criteria of "roentgenographic findings associated with the symptoms of cough, hemoptysis and dyspnea with progressive cyanosis" are hazardous. It can be suspected, but not until an exhaustive work-up is done, with the elimination of all other diseases giving a like picture—and then, only with microscopic section—can a definite diagnosis be made. This disease must be differentiated from the rather benign disease described by Chaves and Abeles,[19] which is found on roentgen film in apparently healthy persons.

It is suggested that lung biopsy, as has been demonstrated by Silverman and Talbot,[12] Kirshner and co-workers,[15] and Crow, Stofer, and Wedin,† may well be done early in all patients with a picture suggesting this syndrome. Whether this will be the answer so far as the clinician is concerned, only repeated biopsies will tell—at least, it will give him a chance to use corticotropin and cortisone early in the disease, as has been suggested.

*Etiology.*—On the basis of their own findings, Hamman and Rich concluded that either a virus or a chemical irritant might be the cause. From a study of the literature, Rubin and co-workers [20] were impressed with the fact that the condition behaves like a pneumonia. Histologically, in many cases the picture resembles influenzal pneumonia, with a characteristic alveolar hyaline membrane. However, the wide-spread and progressive fibroblastic proliferation is absent in the usual viral pneumonitis. While some of Hamman and Rich's cases, and the majority of other cases, showed eosinophiles, lymphocytes, and plasma cells in the alveolar septa,

† Crow, E. W.; Stofer, B. E., and Wedin, P. H.: Acute Diffuse Interstitial Fibrosis of the Lung: Case Report, personal communication.

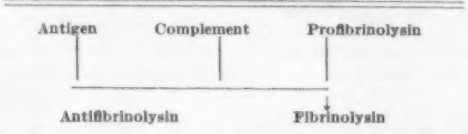
Callahan and co-workers [11] and Golden and Bronk [14] did not see any inflammatory cells in the interstitial tissue. The case of Kneeland and Smetana [21] manifested lesions compatible with periarteritis nodosa in the pulmonary arterioles, while that of Katz and Auerbach [22] showed contracture deformities and atrophy of the hands resembling scleroderma or dermatomyositis.

Of great interest are the observations of Morrow, Schroeder, and Perry,‡ that hexamethonium and hydralazine (Apresoline) hydrochloride have given rise to fatal interstitial pneumonia in 5 of 89 cases of malignant hypertension. The condition was identical with acute interstitial fibrosis of the lungs. However, in four cases the disease progressed rapidly to death within a week, without development of cor pulmonale. According to these authors, it is not known whether the disease was caused by a viral infection superimposed upon pulmonary dynamics altered by hexamethonium, chemical irritation due to hydralazine hydrochloride, or the interaction of both agents on pulmonary tissue.

Dr. E. D. Freis § in a personal communication reported on several patients dying with interstitial pneumonitis. These patients had received hydralazine hydrochloride and hexamethonium. Doniach, Morrison, and Steiner [23] reported on lung changes during hexamethonium therapy for hypertension in 3 out of 54 hypertensive patients. Two patients died. The lung lesion was found to be an organized fibrinous pulmonary edema. This edema was attributed to attacks of left heart failure, probably modified by intermittent lowering of the blood pressure by hexamethonium. The English authors did not consider the lung lesions directly attributable to hexamethonium therapy.

If we accept the view that the process represents a series of attacks of interstitial pneumonitis, with inflammatory fibrosis following each attack, the question arises why the inflammatory fibrinous exudate in the alveolar septa does not become resorbed, but becomes organized. It is the same problem which presents itself in understanding carnification of the lung following lobar pneumonia. Except for formation of fibrous plugs in alveoli, carnification of the lung has anatomically much in common with diffuse interstitial fibrosis. Also, in carnification following lobar pneumonia, the alveolar walls become thicker and fibrous. The alveolar cells become cuboid to columnar, forming glandular, often irregular, structures. Why in rare cases of lobar pneumonia resolution and resorption of inflammatory exudate fail to occur has never been satisfactorily explained. Buechner states that the fibrin of inflammatory exudate has to be liquefied to become resorbed; if it becomes hyalinized, fibroblasts invade the fibrinous masses and organize it by collagenous fibers.

TABLE 2.—*Fibrinolysis*




During the resolution of pneumonic exudate, the lung contains an unusually high content of fibrinolytic enzyme. It seems probable that fibrinolysin in plasma or lung tissue is liberated by inactivation of antiplasmin. Fibrinolysis is apparently a very complex enzymatic process in which at least four chemical substances are involved (Table 2). Blocking between two links of the chain will prevent fibrinolysis, and proliferation of fibroblasts will result. The resolution of fibrinous inflammatory exudate and the formation of granulation tissue, according to Selye,[20] is under hormonal control. In animal experiments, cortisone and corticotropin can prevent fibroplasia, while other hormones will stimulate it. In this connection, an interesting observation is that hexamethonium may produce pulmonary fibrosis.

## THERAPY

Regarding therapy, we have nothing to add. Our patient deteriorated rapidly. We

‡ References 23 and 24.

§ Freis, E. D.: Personal communication.

did not use cortisone or corticotropin, and antibiotics seemed to have had no effect on the downhill course. In those reported cases in which cortisone and corticotropin have been used, the results have been consistently disappointing (Table 4). Peabody saw retarded fibroblastic proliferation in the early cases after cortisone administration, but it could not dissolve the fibrous tissue previously laid down in the chronic cases. Therapy, if it is to be effective, must be instituted early. He attributes violent exacerbation and death of his patients to withdrawal of, or reduction in, cortisone and corticotropin therapy. The mechanism of this response remains uncertain. Peabody concluded that these endocrinal agents should be given on a permanent maintenance dose. Should withdrawal be contemplated, the utmost caution is urged.

TABLE 3.—*Pathological Data on Reported Cases of Interstitial Pulmonary Fibrosis*

| Author | Fibrin Intra-alveolar | Intra-alveolar Fibroplasia | Interstitial Fibrosis |
|---|---|---|---|
| Hamman and Rich | .. | + | + |
| Hamman and Rich | + | + | + |
| Hamman and Rich | + | ++ | + |
| Hamman and Rich | + | + | + |
| Eder and others | + | + | + |
| Potter and Gerber | + | + | + |
| Beams and Harmos | + | + | + |
| Peabody and others | .. | + | + |
| Ferrari and others | .. | .. | + |
| Ferrari and others | .. | .. | + |
| Peabody and others | + | + | + |
| Golden and Tullis | .. | .. | + |
| Golden and Tullis | .. | + | + |
| Rubin and others | + | .. | + |
| Golden and Bronk | .. | .. | .. |
| Golden and Bronk | .. | .. | .. |
| Golden and Bronk | .. | .. | .. |
| Kirshner | .. | .. | + |
| Heppleston | + | .. | + |
| Cox and Kohl | + | + | + |
| MacMillan | .. | .. | + |
| Pokorny and Hellwig | + | + | + |

TABLE 4.—*Effect of Cortisone or Corticotropin Therapy on Interstitial Pulmonary Fibrosis*

| Author | Fibrin Intra-alveolar | Intra-alveolar Fibroplasia | Interstitial Fibrosis | Cortisone or Corticotropin |
|---|---|---|---|---|
| Callahan and others | ++++ | + | + | + (24 days before death) |
| Silverman and Talbot | + | .. | + | + (Died) |
| Peabody and others | + | .. | + | + (Died after termination) |
| Peabody and others | + | .. | + | + (Died after termination) |
| Peabody and others | + | .. | + | + (Died) |

## CONCLUSIONS

A case of diffuse interstitial fibrosis of the lungs is reported, with clinical and pathological features similar to those reported by Hamman and Rich.

The disease can be best explained on the basis of one or more attacks of acute interstitial pneumonitis which result in organization instead of resolution. Disturbance of fibrinolysis may explain diffuse interstitial fibrosis better than the assumption of a specific virus or chemical agent.

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# Results of Intraperitoneal Injection of Barium Sulfate Contrast Medium

**An Experimental Study**

**SAUL KAY, M.D.**
**and**
**SUN HAK CHOY, M.D., Richmond, Va.**

Personal experiences * with human tissue reactions to barium sulfate contrast medium have prompted this experimental study in order to determine what might be the effect of barium injected into the peritoneal cavity under prolonged observations. Previous animal experiments with this element appear to be limited to three reports, namely, those of Himmelmann (cited by Strasser [3]); Huston, Wallach, and Cunningham,[4] and Kleinsasser and Warshaw.[5] Huston and co-workers [4] confined their experiments to the instillation of barium into the respiratory tree of rats, while Kleinsasser and Warshaw [5] utilized the peritoneal cavity of six dogs by injecting 20 to 150 cc. of barium sulfate.

Since the dog experiments seemed limited as to both the number of animals employed and the length of observation, it was elected to use white mice (a far less expensive animal) and carry the period of observation up to six months. As the normal life span of mice is approximately 2 years, a 6-month period might correspond roughly to 20 years in the human.

## MATERIAL AND METHODS

Twenty-one Swiss albino male mice were used, 3 to serve as controls. They were approximately 9 weeks old, with an average weight of 32 gm. In groups of three, the mice were injected with a barium suspension into the peritoneal cavity. The mice in each group were administered 1 cc., 2 cc., and 3 cc., respectively. The control group had the same doses of sterile saline. The barium mixture was prepared similarly to that used for routine barium enema roentgenography. After the suspension was made, it was placed in 60 cc. capped bottles and sterilized by heating in an oven for 30 minutes at 160 C. Sterility was checked by bacteriological methods prior to use of the barium. It was planned to kill each group of animals at monthly intervals, the experiment terminating at six months after injection. Unfortunately some animals died at unexpected times, and the greatest mortality occurred within the first eight days, when five animals died. Four of these five had been injected with 3 cc. of barium, and it is entirely probable that death was due to the size of the injection, since it is known that the injection of only sterile saline into the peritoneal cavity of mice may be quite shocking. Two mice injected with 1 and 2 cc. of barium, respectively, wandered from the cage and were lost. The remaining 19 mice were examined at autopsy after spontaneous death or killing at planned intervals, as described above. The presence of adhesions was noted, as well as intestinal distention, and all visceral organs of the abdominal cavity were removed and fixed in Zenker's fluid. Diaphragmatic and abdominal muscles, as well as peritoneum, were utilized, also as indicated. Paraffin sections were routinely prepared and stained with hematoxylin and eosin.

## RESULTS

Barium tended to be lumped into masses and localized within the peritoneal cavity (Fig. 1). In general it was more abundant around the genital organs. Fine adhesions associated with barium granulomata could be noted as early as four days after injection. As a rule, the severer tissue reactions were noted with the larger quantities injected. Intestinal and bladder distention likewise were prominent features with the larger doses.

From the Department of Surgical Pathology, Medical College of Virginia.

* References 1 and 2.

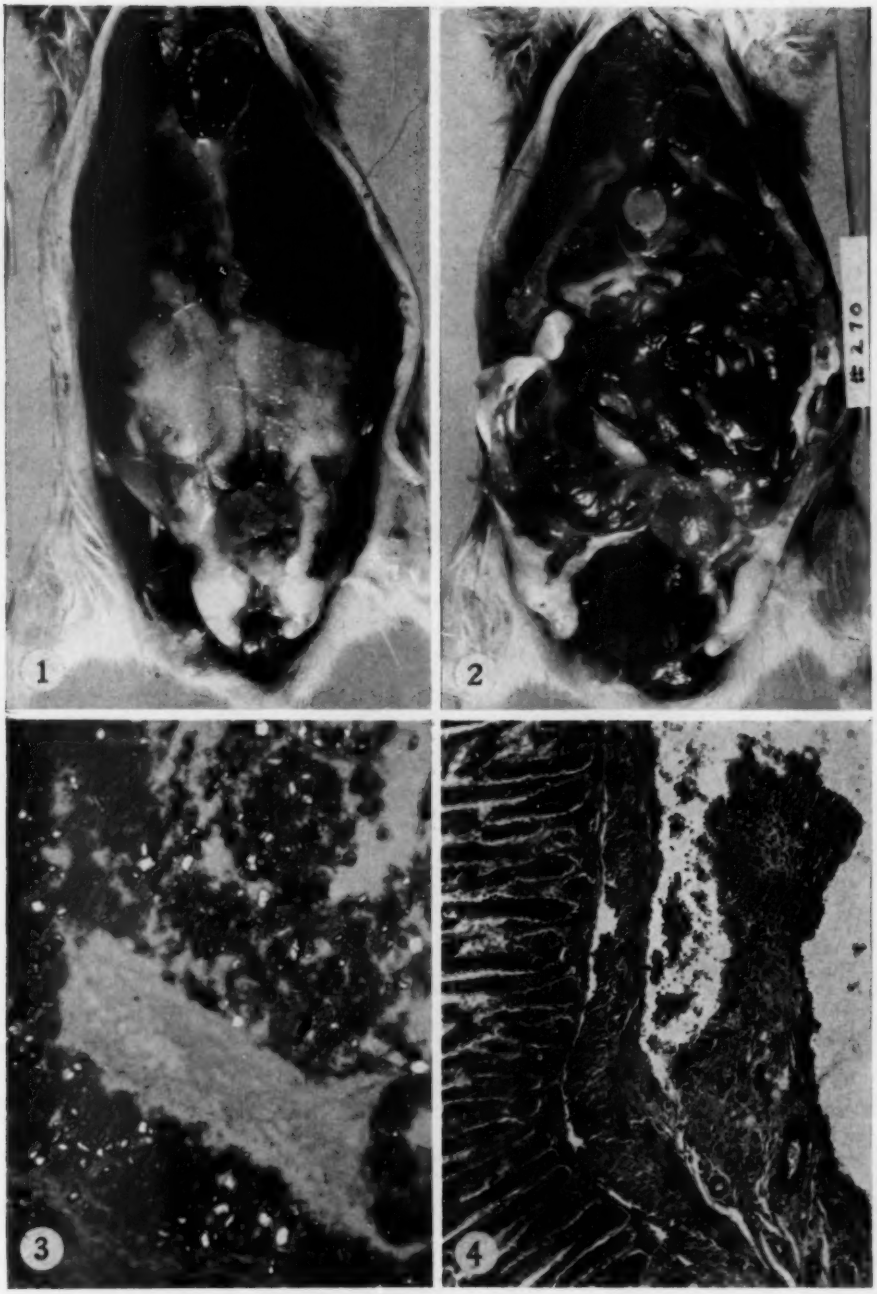


Fig. 1.—Pools of white barium easily identified through intact and transparent parietal peritoneum. Mouse No. 270 killed at six months after 2 cc. injection of barium.

Fig. 2.—Same mouse as in Figure 1, viewed after exposure of peritoneal cavity. Note fine adhesions between viscera and pooling of barium, most marked around genitals.

Fig. 3.—Clumps of barium viewed under polarized light. Mouse No. 301 at eight days after injection of 3 cc. of barium.

Fig. 4.—Mouse No. 267 four days after injection of 3 cc. of barium. Small barium granuloma, chiefly histiocytic, covering serosa of small intestine.

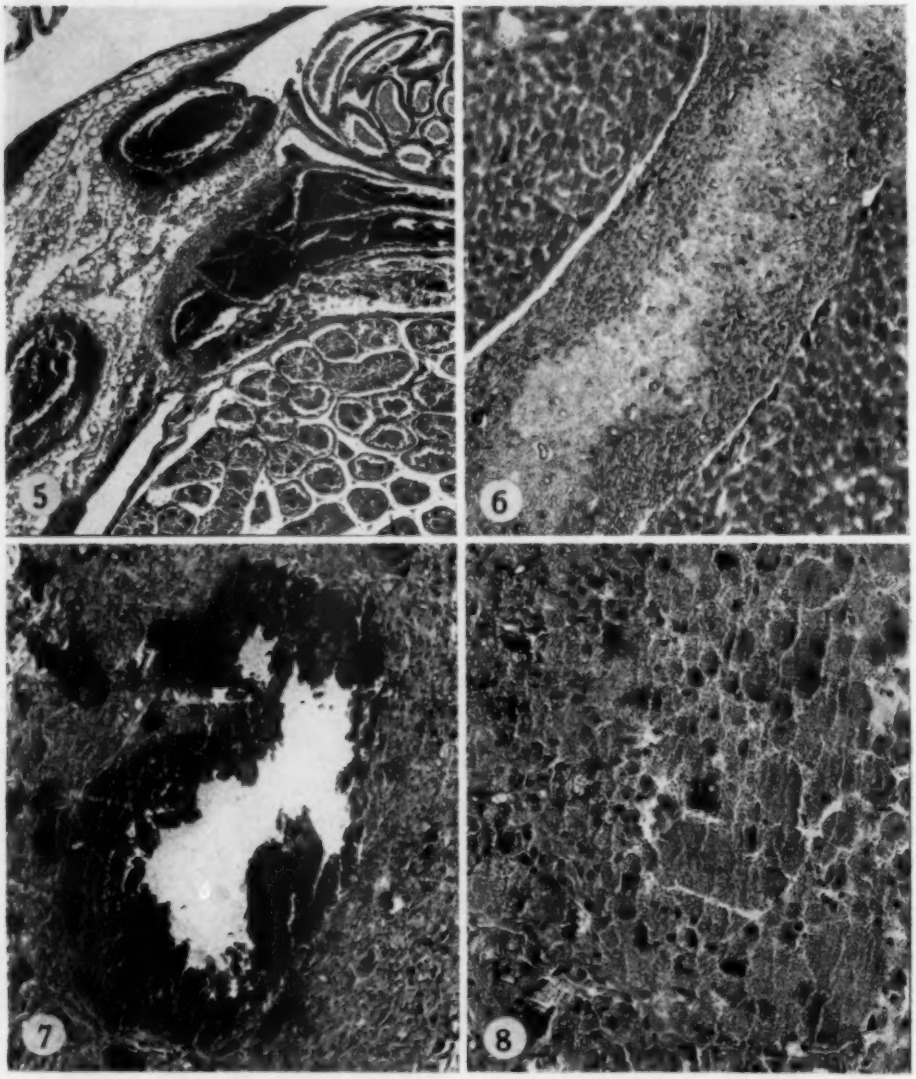


Fig. 5.—Mouse No. 301 eight days after injection of 3 cc. of barium. Exudative type barium granuloma surrounding testis and epididymis. Note dark clumps of barium.

Fig. 6.—Mouse No. 302 two months after injection of 3 cc. of barium. Fibrous granulomatous reaction between liver lobules. Note coarse plates of barium crystals.

Fig. 7.—Mouse No. 280 four months after injection of 2 cc. of barium. Large granuloma surrounding testis, with central necrosis and calcification.

Fig. 8.—Mouse No. 282 four months after injection of 3 cc. of barium. Granuloma around testis and epididymis. Note multinucleated giant cell in center of field.

Gross changes consisted principally of adhesions between loops of bowel, between adjacent viscera, and between parietal peritoneum and intra-abdominal organs (Fig. 2). The adhesions varied from fine velamentous bands, which could easily be broken, to firm, thick strings clinging tenaciously to and between the viscera, rendering separation

difficult. While intestinal distention was present in some animals, none exhibited symptoms or signs of intestinal obstruction.

Microscopically, barium crystals were easily identified in the tissues. When arranged compactly, they imparted a yellowish-brown color to the section. When their presence was in doubt, smaller amounts of barium could be identified with the aid of polarized light, since barium particles are doubly refractile (Fig. 3).

Small but definite granulomas could be found as early as the fourth day after injection. These were noted especially around the epididymis and covering the small intestine (Fig. 4). The granulomas were chiefly histiocytic in type, with coarse and fine plates of barium crystals between the cellular elements. By the eighth day an early fibrous reaction associated with polymorphonuclear leucocytic and lymphocytic infiltration was added to the granuloma (Fig. 5). By the third week the fibrous reaction was the chief finding and inflammatory infiltration was minimal. At two months the fibrous reaction was fairly well developed, and, grossly, adhesions were sometimes rather firm between the visceral organs (Fig. 6). The presence of intestinal distention, however, was noted only with the largest dose.

Four months after injection additional changes included necrosis and calcification of some of the granulomas (Fig. 7). Foreign-body-type giant cells also became evident, and some of these contained barium crystals within cytoplasm (Fig. 8). One mouse (No. 282) showed the presence of barium within lumbar lymph nodes (Fig. 9). This element must have been transported in histiocytes from a surface reaction via lymphatic channels. While some of the larger granulomas revealed central necrosis, never was true tubercle formation seen. At six months the fibrous reaction was mature, and portions of the stroma were hyalinized (Fig. 10). No animal injected with 3 cc. was available at this stage. The 1 cc. mouse (No. 262) had only fine and minimal adhesions, without intestinal distention. The 2 cc. mouse (No. 270) showed firmer and more frequent adhesions, and intestinal dilatation was moderate.

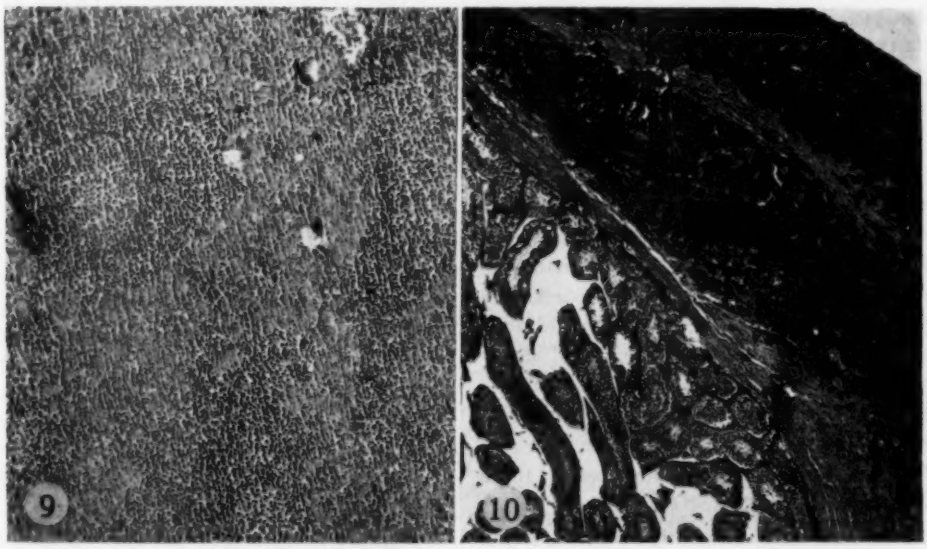


Fig. 9.—Same animal as in Figure 8. Lumbar lymph node containing diffuse collections of barium generally distributed within sinusoids.

Fig. 10.—Mouse No. 270 six months after injection of 2 cc. of barium. Mature fibrous granuloma covering testis. Note hyalinization of stroma surrounded by dark masses of barium.

## COMMENT

The use of laboratory animals in drawing conclusions about what may happen to human tissues under similar circumstances admittedly has its limitations. Even the dog, with its physiologic pattern similar to that of man, has been known to react differently from the human under the impact of experimental investigations. Nevertheless, we have seen the microscopic picture of human tissue reaction to barium when this element was inadvertently introduced into the peritoneal cavity,† and we know that at least the early changes are similar to those recorded here in mice. It has been our desire to learn what would be the effect of unusually large amounts of barium in the peritoneal cavity over a long period of observation.

On a comparative weight basis, 1 cc. of injection into mice is equivalent to more than 2000 cc. of material in man. Since it is the custom to administer about 1 qt. (0.95 liter) of barium suspension to patients requiring barium enema studies, theoretically any amount over 1000 cc. which could accidentally gain access to the peritoneal cavity would be most unusual. The 1 cc. amount is also equivalent to more than 200 cc. in dogs, as used in the experiments of Kleinsasser and Warshaw.[5] The highest amount used by these authors was actually 150 cc.

The maximum observation period for the mice was 6 months, which is equivalent to about 20 years in man. As can be seen from our results, adhesions could be produced in all animals, but these tended to be fine and easily separable in animals injected with 1 cc. Similarly, intestinal distention was insignificant in animals given 1 cc. The severest reactions occurred in animals given 3 cc., an amount equivalent to more than 6 qt. of barium in the human. Even with this dose, one mouse was alive at four months before killing, with signs only of intestinal distention. An animal given 2 cc. showed only moderate intestinal distention at six months but seemed otherwise perfectly normal.

† References 1 and 2.

A final problem, as yet unsettled, still remains. What methods, if any, short of surgical intervention, do we have at our disposal for the removal of barium from the peritoneal cavity? While the presence of barium per se is probably perfectly compatible with good health, with no deleterious complications resulting therefrom, a simple method of extracting this element would be desirable. The answer to this question must await further investigation.

## SUMMARY

Experimental observations following the injection intraperitoneally of large doses of sterile barium suspension in mice are recorded. The basic response to the injected material is a granuloma, noted as early as the fourth day, which is chiefly histiocytic in type. The granuloma becomes more progressively fibrous in nature as the period of observation increases.

Rather dense fibrous adhesions may be produced between loops of intestine and surrounding viscera with larger barium doses. The ultimate effects appear to be nondeleterious to the host aside from intestinal distention, which is severe only with the largest dose employed.

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# News and Comment

## GENERAL NEWS

**Conference of Chicago Medical Society.**—At the annual clinical conference of the Chicago Medical Society, held March 1-4, Dr. Frank J. Dixon, of Pittsburgh, presented a paper on a study of 1000 testicular tumors, and Dr. John R. Schenken, of Omaha, discussed the problem of cystic ovary.

**Lecture on Cancer Chemotherapy.**—Dr. Cornelius P. Rhoads, Director of the Sloan-Kettering Institute for Cancer Research, New York, gave the 9th annual lecture of Tau Chapter of Phi Delta Epsilon fraternity, at Syracuse University, on Feb. 15. He discussed "Present Status of Cancer Chemotherapy."

**Seminar on Renal Diseases.**—The Michigan Pathological Society and the North Central Region, College of American Pathologists, held a joint session at Henry Ford Hospital on Feb. 12. Dr. Arthur C. Allen, of New York City, served as moderator for a slide seminar on Renal Diseases, and the following program was presented at the meeting:

Kidney Function in Disease, Arthur C. Corcoran, Cleveland; Don Carlos Hines, Indianapolis, and Irvine H. Page, Cleveland.

Renal Physiology in Obstructive Uropathy, Jack Lapides, Ann Arbor, Mich.

Effect of Increased Work-Load on the Kidneys, Frank W. Hartman, Detroit.

Biochemical Changes in Uremia on the Artificial Kidney, Lloyd T. Iseri, Detroit.

Renal Aspects of the Pathogenesis of Hypertension, Irvine H. Page, Cleveland.

## PERSONAL

**Beaumont Lecture by Dr. Carl V. Weller.**—Dr. Carl V. Weller, Professor of Pathology, University of Michigan Medical School, delivered the Beaumont Lecture before the Wayne County Medical Society on Feb. 7. He discussed the problem of causation of cancer of the lung.

## DEATHS

**Dr. Paul Brindley.**—Dr. Paul Brindley, Chairman of the Department of Pathology of the University of Texas, died on Dec. 28, 1954.

**Dr. Joseph Felsen.—**Dr. Joseph Felsen, director of laboratories and research at the Bronx Hospital, New York City, died on Jan. 27, 1955.

# Books

**Arrest of Bleeding: Physiology, Pharmacology, Pathology.** Jacques Roskam, M.D. Price, $2.75. Pp. 71, with 21 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1954.

The author of this monograph is probably the most active European investigator of hemorrhagic problems. Here he presents the results of almost thirty years of study in a manner that, while evaluating the various factors that influence hemorrhage and its control, emphasizes the interrelationships of these mechanisms. The outstanding contribution of the volume is the introduction of techniques for measuring hemorrhage that permit statistical analyses. This is a marked advance in a field which has heretofore been confused because of inadequate methods.

Techniques and methods are first considered by which hemorrhage can be studied and evaluated. Secondly, the various physiological aspects of hemorrhage are considered, and, then, the pharmacological agents affecting hemostasis are discussed. The final portion of the monograph concerns hemorrhagic diseases and the treatment of hemorrhage and thrombosis.

Several important observations are again emphasized by the author. The importance of local vasoconstriction in spontaneous hemostasis is supported by experimental observations. The mechanisms of epinephrine modification and shortening of bleeding times are investigated and felt to be due to oxidation products. The synergistic effects of heparin-type substances and vasodilation in increasing bleeding times are pointed out.

It is unfortunate that this monograph suffers in translation, so that sentence structure is often cumbersome and terminology somewhat different from that in common usage. Also, the pharmacological discussion of hemostatic agents considers some substances not in common use in this country, while ignoring several drugs, notably protamine, toluidine blue, and vitamin $K_1$ oxide, which are used. However, in spite of these slight handicaps, the monograph is valuable for its physiological approach to hemorrhagic problems and, in particular, for its statistical advances in these problems.

**Handbook of Tropical Dermatology:** Volume II. By R. D. G. Ph. Simons. Price, $15.00. Pp. 856, with 511 illustrations. Elsevier Press, Inc., 118 Spuistraat, Amsterdam C, Netherlands, 1953.

Volume II of "Handbook of Tropical Dermatology" consists of contributions from over fifty authorities, representing practically all parts of the world. The opening section, concerning diseases due to animal parasites, is comprehensive and includes entities ranging from common scabies through lesions from all varieties of fleas and mites, dramatic black widow spiders, tarantulas, and scorpions, to such regional curiosities as harara of the Israeli area, and including even the bite of dubiously pathogenic mite, Demodex folliculorum.

Over 400 pages are devoted to medical mycology, some of which specifically concern tropical mycology, but most of which are devoted to a survey of general mycology. The chapter concerning mycological technique is well presented, in a practical and detailed way.

The 100 pages devoted to benign and malignant tumors of the skin also contain many special references to tropical peculiarities, especially as regards occupation, habits, skin color, and exposure to sunlight, but, for the most part, comprise a general review of skin tumors which are well represented in all climates of the world.

There are interesting chapters on the curious entities of ainhum, porphyria (as it exists in South Africa), and "Brazilian pemphigus."

Though little is mentioned about morbid pathology, the information about subjects covered is otherwise quite detailed and accompanied by adequate bibliographies.

The volume seems larger than it needs to be for the purpose which it serves—a handbook of tropical dermatology. The multiplicity of authors gives a refreshing variety of style throughout but also serves to create considerable overlapping and, in some instances, a rather awkward development of large topics. Also, one might take exception to dogmatic statements in rather controversial issues. However, this volume, in addition to Volume I, is an excellent reference for those interested in the entities of tropical dermatology.

**Gerichtliche Medizin.** By Dr. Gerhard Hansen. Price, 19.50 German marks. Pp. 240, with 125 illustrations, some in color. Georg Thieme, Herweg 63, (14a) Stuttgart, 1954.

This volume is intended primarily for orientation of the medical student in the field of forensic medicine. It represents some revision of previous material necessitated in part by changes in postwar German law. The first portion is devoted to general considerations of legal medicine, particularly the relationships of the coroner, pathologist, and lawyer. Much of the first portion concerns the signs of death and determination of time of death. Most of this material is well known and some is outmoded; only a short discussion of various enzyme reactions is given. Much space is devoted to various types of decomposition of the body under varying circumstances. The section ends with a consideration of the mechanisms of sudden death. Most of this material is elementary and some definitely outdated, such as the concept of status thymicolymphaticus.

The various special problems of legal medicine are considered in the second portion. The approach to problems of death under unusual circumstances is first discussed, followed by sections on injuries inflicted by blunt and sharp instruments, by shooting, by physical agents, and by strangulation. Toxicological problems are dealt with in sixteen pages and this discussion is most superficial and incomplete. The volume concludes with short chapters on abortion, infanticide, and some of the legal problems of psychiatry.

This monograph is superficial and much of the material is outdated; some concepts presented are definitely erroneous. It is illustrated only with gross photographs, many of which could better be classed as horror pictures and which contribute nothing to the presentation. No photomicrographs are included and there is almost no discussion of histopathology. While the book may have some merit as an introduction to forensic medicine for the German medical student, it is of no value to the practicing pathologist in this country.

**Proceedings of the Second National Cancer Conference, 1952.** Price, $7.50. Pp. 1,687, in two volumes, American Cancer Society, Inc., 47 Beaver St., New York 4, 1954.

The Second National Cancer Conference, jointly sponsored by the American Cancer Society, Inc., the National Cancer Institute of the United States Public Health Service, and the American Association for Cancer Research, was held in Cincinnati on March 3 to 5, 1952. The proceedings are now published in these two volumes. Here are found the introductory remarks; ten papers given at general sessions; two round-table discussions; and separate panel meetings on cancers of the breast, genitourinary tract, head and neck, female genital tract, gastrointestinal tract, and lung, on lymphoma-leukemia, and on radiobiology, genetics, cytology, chemotherapy, virology, steroids, and isotopes. The volumes are indexed by author and subject. The purpose of the meeting was educational—for practitioners and investigators. It is impossible to review these two volumes in detail, but the coverage of subject matter is wide, and the numerous contributors write with authority. These proceedings admirably present the currrent status of many cancer problems in the clinic and laboratory.

**Textbook of Pediatrics.** Edited by Waldo E. Nelson, M.D. Sixth Edition. Price, $15.00. Pp. 1581, with 478 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1954.

This sixth edition of the "Textbook of Pediatrics," edited by Waldo E. Nelson, represents the combined contributions of many distinguished pediatricians. An attempt has been made to reduce the size of the book as much as possible, but obviously this is a very difficult problem with this type of textbook. The book is profusely illustrated, with a number of colored plates. Many of the references are to recent developments, and the indexing is excellent. This book will continue to be of value to pediatricians and to medical students concerned with the general problems of pediatrics.

**Chemotherapy of Infections.** By H. O. J. Collier, B.A., Ph.D., M.I.Biol. Price, $4.00. Pp. 248, with 53 figures. John Wiley & Sons, Inc., 440 4th Ave., New York 16, 1954.

This little book contains a great amount of timely material dealing with the development of chemotherapeutic and antibiotic treatment. The author is Chief Pharmacologist of Allen and Hanburys Ltd. A foreword by Sir Alexander Fleming gives a brief résumé of the development of chemotherapy. In the sixteen chapters the author deals with the fundamental principles of chemotherapy and antibiotics and with the development of various antibiotics and chemotherapeutic materials. He emphasizes particularly the historical aspects of the various subjects and in

each chapter has from ten to twenty recent well-selected references. By means of more than fifty illustrations and over twenty tables much information is furnished in a compact way. The attempt has apparently been made to present the information in as simple a form as possible. Because of that the early chapters dealing with problems of immunology are necessarily quite briefly handled. For the most part, however, the emphasis on the various drugs and their mode of action is clear and lucid. The book should be of value and interest to all concerned with the current problems of chemotherapy and antibiotics.

**Cancer: Diagnosis, Treatment, and Prognosis.** By Lauren V. Ackerman, M.D., and Juan A. del Regato, M.D. Second Edition. Price, $22.50. Pp. 1201, with 702 illustrations and 5 color plates. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1954.

The first edition of this volume, which appeared in 1947, has found considerable acceptance, particularly among surgeons in various phases of their training. The criticism that no single book can hope to cover such a complex subject is unjustified, as the authors make no pretext of completeness. This edition, like the first, is intended to provide "information on the treatment and prognosis not available in treatises on pathology . . . with an integrated view of all of these aspects."

The first section of the book is devoted to subjects of general interest and application in the field and covers such topics as cancer research, pathology of cancer, surgery of cancer, and radiotherapy of cancer. The second portion considers tumors of the various systems. Though the primary consideration is that of malignant tumors, benign tumors are included when necessary from a diagnostic or prognostic point of view. Each chapter considers anatomy, incidence and etiology, pathology, clinical evolution, diagnosis, treatment, and prognosis of tumors of the various systems.

This second edition has been considerably revised, and some chapters have been entirely rewritten; references have been brought up to date. The book can not be recommended to pathologists for its pathological descriptions of the various tumors; such descriptions are necessarily too brief. However, the volume is to be enthusiastically recommended to pathologists for its approach to the problems of treatment and prognosis. An appreciation of the practical aspects of cancer should be a part of every pathologist's training. This book offers such a point of view.

**The Hidden Causes of Disease.** By Antonio Benivieni; translation by Charles Singer; Biographical Appreciation by Esmond R. Long. Price, $6.75. Pp. 217, with 5 plates. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1954.

All physicians interested in the beginnings of modern medicine will welcome this beautifully printed book, in which the original text is carried on one page and the translation by Charles Singer on the opposite. As Long says in his section dealing with Benivieni's contribution to pathological anatomy, the book is to be commended for "the spirit of the effort" to study disease objectively at a time when humoral pathology dominated medical thinking. More than one hundred case histories are included, with about fifteen necropsy protocols. A reading of these enables one better to understand the state of medicine in Italy some three centuries before the time of Morgagni. To quote Long again, "It was the beginning of the end of the old humoral tradition." Although the necropsy reports are often inadequate, they, nevertheless, "mark a milestone in the history of medicine." Benivieni was essentially a clinician, but he is probably rightly regarded, in the words of Cruveilhier, as "the father of pathological anatomy." All students of medical history will be grateful to Charles Singer and Esmond R. Long, and to Charles C Thomas, for their collaboration in this effort.

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Pascale, L. R., and Olwin, J. H.: Circulation 9:230 (Feb.) 1954.

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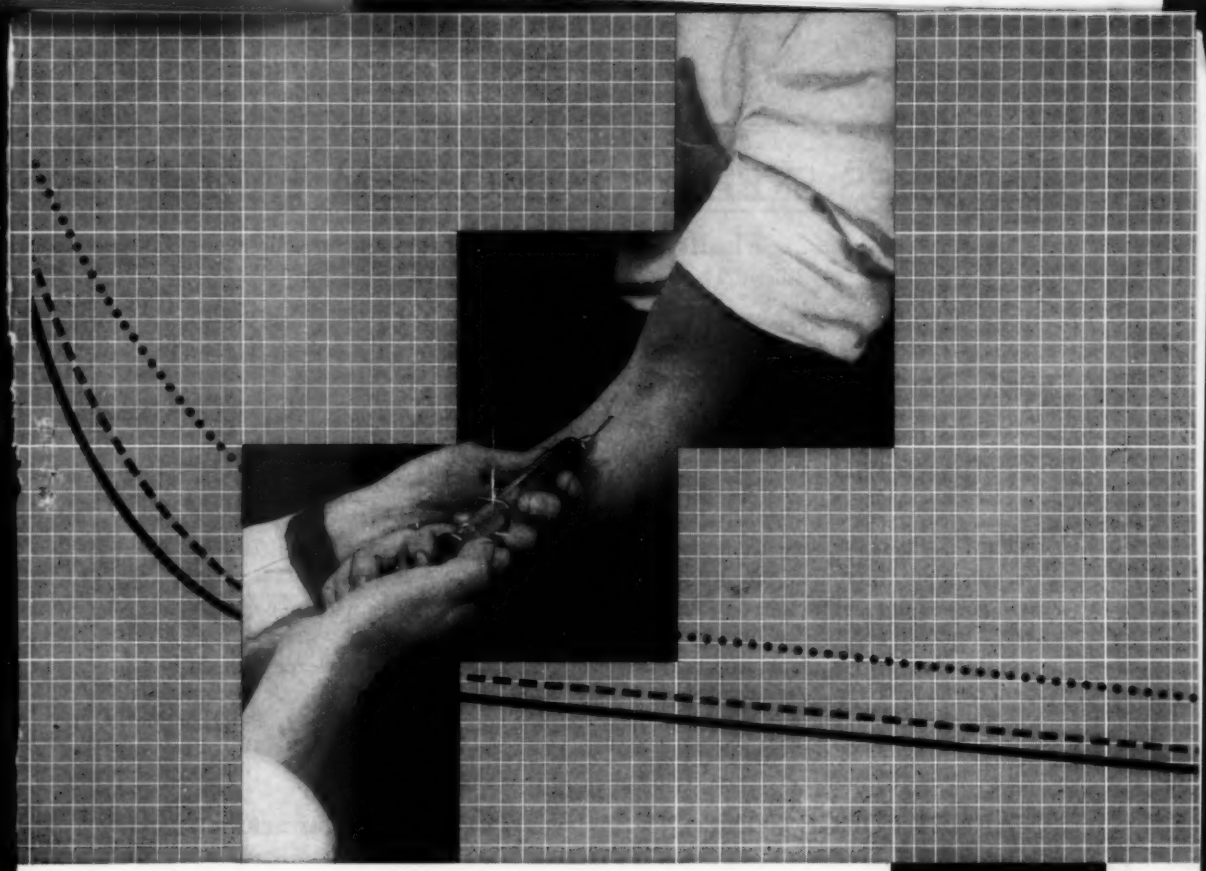

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1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. 222:207 (Aug.) 1951.

2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey 48:1 (Jan.) 1951.

3. Shapiro, S., *et al.*: Am. Heart J. 40:766 (Nov.) 1950.

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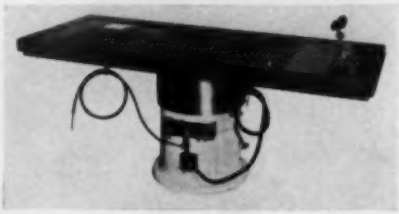

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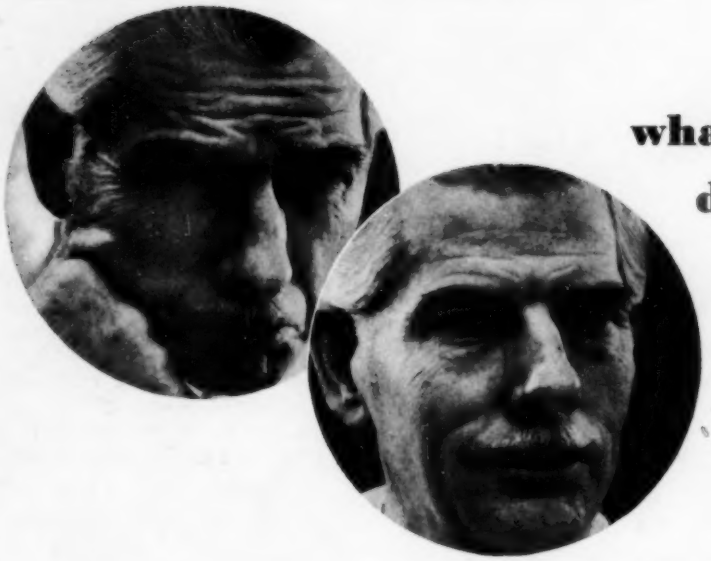

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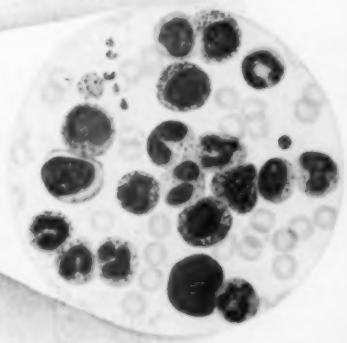

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